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ABSTRACT BOOK

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ABSTRACT BOOK

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The European Hematology Association (EHA) aims to promote excellence in clinical practice, research and education in European hematology.
EHA was founded in June 1992 and today – with over 3,000 members from 100 countries – is a consolidated representative of European hematologists.

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- Become the official European representative of hematology and hematologists – especially where research, education and regulatory issues are concerned – and to become a conduit for European harmonization.
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- Reach out and offer a platform to countries that wish to further develop excellence in hematology.
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- Exchange and disseminate knowledge and scientific information in the field of hematology.

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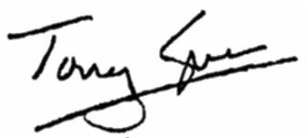
Word of welcome

On behalf of the EHA Board and the Scientific Program Committee we are pleased to present the Abstract Book of the 17th Congress of EHA.

The Scientific Program Committee has compiled an exciting and topical program of Simultaneous Sessions and Poster Sessions from over 2,000 submitted abstracts. Please join our expert moderators for a walk along the posters in your field of interest on Friday and Saturday. The six best abstracts have been selected for presentation during the Presidential Symposium on Saturday, June 16.

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



Tony Green
Chair Scientific Program Committee

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POSTER SESSION I

Acute lymphoblastic leukemia - Biology I

0001

DIFFERENTIAL CHEMOKINE RECEPTORS EXPRESSION IN RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): A ROLE FOR CXCR3 IN RELAPSES

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Background. Leukemic relapses remain the major cause of treatment failure in childhood acute lymphoblastic leukemias (ALL). Resistance to chemotherapy may be intrinsic to lymphoblasts, but it is also possible that the environment may minimize the effects of antileukemic drugs. Leukemic blasts may infiltrate areas where the levels of chemotherapies are suboptimal. In addition, the environment may provide prosurvival signals to the leukemic cells. There is scant information on the molecular basis of these processes. Identifying molecules that allow the leukemic cells to localise and survive in special niches may help in finding new targets for those patients at high risk for relapses. **Aims.** To assess whether chemokines expressed by ALL blasts may have a role in relapses. **Methods.** We compared the levels of chemokine receptors in marrow samples from 94 children with ALL, under an approved protocol. Clinical data were collected from each patient for statistical analysis. Eighty-two samples were drawn at the time of diagnosis, and 15 at relapse. Only 3 patients had samples corresponding to diagnosis and to relapse. We quantitated the levels of chemokines in central system fluid (CSF) samples by ELISA or by Bioplex assays. The functional role of specific chemokines was studied *in vitro* and *in vivo*. Chemotaxis was assessed in standard transwell assays. The influence of chemokines on chemotherapy-induced apoptosis was assessed by flow cytometry, western blotting and caspase activity assays. Immunodeficient mice transplanted with human leukemia cells were treated with a specific antagonist to test the effects of inhibition *in vivo*.Results. Leukemic blasts from patients with T-ALL and marrow relapses expressed CCR5, CXCR3 and CXCR5 at higher levels compared to those who did not relapse. Patients with T-ALL and central nervous system (CNS) relapses expressed higher levels of CCR3, CCR5, CCR7 and CXCR3 compared to those with no CNS relapse. In patients with T-ALL, the levels of CXCL10 (a CXCR3 ligand) in CSF were significantly higher among patients with CNS relapses. Higher expression of CCR5 and CCR8 were found among patients with B-ALL and marrow relapses than among those who did not relapse. Children with B-ALL and CNS relapses expressed higher levels of CCR3, CCR5 and CCR8 in their blasts than those with no CNS relapses. The role of CXCR3 was further studied in functional assays. Signaling through CXCR3 induced chemotaxis in leukemic cell lines and in primary leukemic cells, depending upon the levels of CXCR3 expression. CXCL10 specifically diminished chemotherapy-induced apoptosis on ALL cells expressing CXCR3, partially inhibiting caspase activation and maintaining the levels of the antiapoptotic protein Bcl-2. Finally, immunodeficient mice engrafted with a CXCR3-expressing human leukemic cell line, showed decreased infiltration of marrow, spleen and CNS after receiving a CXCR3-antagonist molecule. **Summary and Conclusions. .** The expression of some chemokine receptors was upregulated upon leukemic relapse, both in B- and in T-ALL, and in cases of medullary and extramedullary involvement. CXCR3 signaling may have a dual function in relapses: chemotactic for the localisation of leukemic blasts in specific niches, and it may also confer resistance to chemotherapy

0002

INHIBITION OF MCL1 AND GLYCOLYSIS SYNERGISTICALLY SENSITIZES TO PREDNISOLONE IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

M Ariës, R van den Dungen, R Pieters, M Den Boer
Erasmus MC - Sophia Childrens' Hospital, Rotterdam, Netherlands

Background. Prednisolone resistance is one of the main prognostic factors of precursor B acute lymphoblastic leukemia (ALL). Overcoming prednisolone resistance would improve survival rates. Recently, we demonstrated that knockdown of the anti-apoptotic gene *MCL1* sensitized leukemic cell lines to prednisolone. We also discovered that 2-deoxyglucose (2-DG), an inhibitor of glycolysis, had the same effect. **Aims.** Because glycolytic and apoptotic pathways are related and tightly regulated survival pathways, we investigated a possible synergistic mechanism of *MCL1* and glycolysis in prednisolone resistance. In addition, we tested a newly developed locked nucleic acid against MCL1 (LNA-MCL1). **Methods.** Leukemic cells of prednisolone sensitive and resistant pediatric ALL patients were treated *in vitro* for 48h with 1 µg/ml or 250 µg/ml prednisolone. Hereafter, protein was isolated and reverse phase protein array was performed. MCL1 knockdown was achieved by lentiviral silencing and LNAs in two different leukemic cell lines and the effect was assessed with RTQ-PCR and Western blot. Cell viability and cell count were analyzed by MACSQuant. Glucose consumption was measured using a glucose assay kit (Sigma). A suboptimal concentration of 0.5 mM 2DG was used to inhibit glycolysis in MCL1 silenced cells. Cytotoxicity of cells towards prednisolone was determined by the *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide drug resistance assay. Statistical Mann-Whitney tests were performed in SPSS. **Results.** Exposure of primary patients' leukemic cells to prednisolone significantly reduced the MCL1 protein levels in prednisolone-sensitive compared to prednisolone-resistant cases ($P<0.05$; 2.6 to 2.9 -fold reduction in sensitive cells). Exposure to 3 LNA-MCL1 antagonists resulted in a highly efficient knockdown of MCL1 protein levels by 82+/-16% which was similar to the silencing of MCL1 by 2 lentivirally delivered shRNAs, 72+/-12%. Silencing of MCL1 in leukemic cells by either LNA-MCL1s or shMCL1s decreased the proliferation rate by 1.9 to 9-fold ($P<0.05$), increased the apoptosis-rate by 1.4 to 4.7-fold ($P<0.05$) and sensitized to prednisolone by 1.8 to 80.8-fold ($P<0.05$). Interestingly, silencing of MCL1 by LNA-MCL1s or shMCL1s both stimulated the consumption of glucose by 1.1-2.5-fold ($P<0.05$), indicating that cells with silenced MCL1 upregulate the glycolytic process to enable survival. In correspondence, inhibition of glycolysis by 2-DG reduced the proliferation rate of MCL1-silenced cells by 1.3 to 3.9-fold ($P<0.05$). Most importantly, silencing of MCL1 and inhibition of glycolysis synergistically sensitized leukemic cells to prednisolone by 1.7+-0.6 fold. **Summary and Conclusions. .** MCL1 is a potent target to therapeutically convert drug resistance in pediatric acute leukemia. However, MCL1 silenced cells upregulate glycolysis to circumvent apoptosis. Silencing of MCL1 and inhibition of glycolysis synergistically sensitized leukemic cells to prednisolone. These data therefore provide evidence for concomitant causes of resistance, and indicate that MCL1 and glycolysis should be targeted simultaneously to modulate prednisolone resistance in acute leukemia.

0003

HIGH-THROUGHPUT FISH SCREENING REVEALS THE ACCURATE INCIDENCE OF IGH@ TRANSLOCATIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

J Russell[1], L Jones[1], A Erhorn[1], C Schwab[1], K Laczko[2], C Harrison[1]
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Chromosomal translocations lead to oncogene activation or gene fusion in hematological malignancies, where they play an important role both in diagnosis and as an indicator of prognosis. Translocations involving the immunoglobulin heavy chain locus (*IGH@)* in B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) have recently been characterised and found to be different from those reported in the mature B-cell malignancies, although their expression is always deregulated due to the juxtaposition of transcriptional enhancers within the locus. The *IGH@* locus rearranges with multiple genes including: the *CEBP* family of transcription factors, cytokine receptors: *EPOR* and *CRLF2,* and the inhibitory transcription factor, *ID4*. We have developed a high-throughput screening approach to ascertain the true incidence of *IGH@*

translocations in BCP-ALL. We have screened an entire childhood clinical treatment trial (ALL2003) using the Vysis LSI IGH break-apart rearrangement probe (Abbott Molecular) with automated scanning using the CytoVision 7.2 SPOT counting system (Leica Microsystems). We optimised slide set up, hybridisation and scanning templates for capture and automated scoring of 200 cells in over 2000 patients. We have identified *IGH@* translocations in 4% (115/2580) of childhood ALL with the translocation positive population ranging from 5% (cut off value of 3%) to 100% of nuclei. A median age of 10 years and white cell count of $11x10^9$/L was observed in patients with *IGH@* translocations. The median age was significantly higher than all patients entered to this trial (5 years). This is consistent with our previous findings of *IGH@* translocations being frequently observed in adolescents and young adults. We identified the involvement of *CRLF2* in 19%, the *CEBP* gene family in 5%, and *ID4* in 6% of the *IGH@* translocations. DNA was available for Multiplex Ligation-dependent Probe Amplification (MLPA) from 43 *IGH@* translocation patients. Copy number alterations were investigated using the MLPA kit P335-A1 (MRC Holland), which includes probes for *IKZF1, CDKN2A/B, PAX5, EBF1, ETV6, BTG1, RB1*, and the PAR1 region: *CRLF2, CSF2RA, IL3RA*. We identified deletions in 40%, 30%, 19%, 7%, 9%, 12% and 9%, respectively. The number of gene deletions ranged from zero (37%), one (28%), two (26%), three (7%) and five (2%) deleted in the same patient. We have also screened an entire adult trail (MRC UKALLXII) for the presence of *IGH@* translocations, identifying 8% (58/690) of patients whose outcome was inferior to other BCP-ALL subtypes. Interestingly, in 70% and 57% of *IGH@* positive children and adults, respectively, the translocation partner remains to be elucidated, suggesting the presence of as yet unidentified cryptic rearrangements. Characterisation of these partners is necessary to identify additional oncogenes that may be involved in leukaemogenesis or provide potential therapeutic targets.

0004

THE TRANSPHOSPHORYLATION OF BCR IN THE RESISTANCE OF BCR/ABL HARBORING THE T315I MUTATION

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Background. In Philadelphia chromosome positive (Ph+) leukemia, the t(9;22) encodes for the BCR/ABL fusion protein. BCR/ABL is a constitutively activated kinase which induces the leukemic phenotype because of the aberrant activation of multiple signaling pathways. Targeted inhibition of BCR/ABL by ABL-kinase inhibitors (AKI) such as Imatinib, Nilotinib or Dasatinib induces apoptosis in BCR/ABL transformed cells and leads to complete remission in Ph+ leukemia patients. However, the “gatekeeper” mutation T315I confers resistance against all actually approved ABL-kinase inhibitors. It seems not only to decrease affinity for kinase inhibitors but to confer additional features to the leukemogenic potential of BCR/ABL. In our previous studies we determined the role of T315I in the resistance and its contribution in the leukemogenic potential of BCR/ABL. We studied its influence on loss-of-function mutants with regard to the capacity to mediate factor-independence. We found that resistance of BCR/ABL-T315I was accompanied by the trans-phosphorylation of endogenous BCR at tyrosine 177. **Aims.** In order to further disclose the mechanisms of resistance mediated by T315I we investigated the role of the transphosphorylation of endogenous BCR. Therefore we studied the effects of T315I in both BCR/ABL mutants lacking functional domains in the BCR portion indispensable for the oncogenic activity of BCR/ABL such as the N-terminal coiled coil (CC), the tyrosine phosphorylation site Y177 and the serine/threonine kinase domain (ST), as well as on the ABL-portion of BCR/ABL (#ABLT315I). **Methods.** All mutants were retrovirally expressed iin Ba/F3 cells and tested for their capacity to mediate factor independence in the absence and presence of Imatinib. The transformation potential was studied in untransformed Rat-1 fibroblasts by focus formation assays and colony formation in semi-solid medium. BCR-expression was targeted by RNA interference. **Results**. Here we report that i.) T315I restored the capacity of loss-of-function mutants of BCR/ABL to confer factor-independence and their transformation potential in Rat-1 cells; ii.) Short hairpin (sh) RNA against endogenous BCR reduced expression of endogenous BCR; iii.) shRNA against endogenous BCR abrogated the capacity of loss of function mutants of BCR/ABL-T315I to mediate factor independent growth of Ba/F3 cells and to strongly reduce their transformation potential in Rat-1 cells. **Summary**. Our data show that the T315I restores the capacity of loss-of-function mutants to transform cells which is dependent on the transphosphorylation of the endogenous BCR suggesting endogenous BCR as a therapeutic target to overcome resistance by T315I.

0005

NOVEL ACTIVATING MUTATIONS LACKING CYSTEINE IN TYPE I CYTOKINE RECEPTORS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Interleukin 7 receptor alpha (IL7R) is required for normal lymphoid development. IL7R can dimerize with cytokine receptor-like factor 2 (CRLF2) to form the receptor to thymic stromal lymphopoietin (TSLP), or with interleukin-2 receptor gamma (IL2RG) to form the receptor to IL-7. We and others have recently reported activating mutations in IL7R and CRLF2 in acute lymphoblastic leukemia (ALL). The addition of a cysteine to the extracellular or transmembrane domains of the receptor, characterizing these mutations activate the receptor by causing ligand- independent receptor dimerization via s-s bonds between the mutated cysteines.Here we report the discovery of a novel type of activating mutations lacking cysteine in IL7R and CRLF2 in ALL. IL7R mutations were found in 3 T-ALL patients and CRLF2 mutation was found in one B-Cell precursor ALL. Three of these mutations were small in-frame indels and one a missense mutation. All were located in the C-terminal region of the transmembrane domain (TMD), in contrast to the more common cysteine insertion mutations, located in the N-terminal region of the TMD. The mutations in IL7R were: 254 insertion (ins) EKV, 254 ins GEA and V253G. The CRLF2 mutation was 244 ins EIM.To decipher the effect of IL7R and CRLF2 ‘non cysteine‘ mutations, we generated interleukin 3 dependent mouse pro-B cells (BaF3) and the mouse IL7 dependent MOHITO cell line that expressed either the wt receptors or the mutated constructs. We demonstrate that the mutated constructs lead to cytokine independent growth associated with phosphorylation of STAT5 and JAK1. As expected, unlike the cysteine mutated protein, no homodimerization was observed in Western Blot under non reducing condition.We have thus identified a novel mechanism for constitutive activation of cytokine receptors in leukemia. Given the presence of similar receptors in multiple cell lineages, such mutations may occur in other neoplastic disorders.

0006

MOLECULAR CHARACTERIZATION OF TP53 MUTATIONS IN B-ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) REVEALS MISSENSE SUBSTITUTIONS, ABERRANT EXON-JUNCTIONS AND INTRON RETENTION EVENTS

I Iacobucci[1], A Ferrari[1], A Lonetti[2], C Papayannidis[1], S Trino[3], C Venturi[1], A Ferrarini[4], L Venturini[4], M Abbenante[1], S Parisi[1], F Cattina[5], S Soverini[1], D Russo[5], M Vignetti[6], F Pane[7], M Delledonne[4], M Baccarani[1], G Martinelli[1]
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Recently, copy number and sequence alterations of *TP53* has been shown in 12.4% of pediatric B-ALL in which they independently predicted high risk of treatment failure (Hof J et al. JCO 2011). Since the pattern, frequency and significance of *TP53* aberrations in adult B-ALL have still to be determined, in this study we set-up a sensitive assay to address this issue.Deletions and uniparental disomy (UPD) involving *TP53* were assessed in 146 DNA samples from adult Philadelphia-positive (Ph+)(n = 126) and Ph-negative (n = 20) ALL by Genome-Wide Human SNP 6.0 array (Affymetrix). UPD events were not detected whereas losses of *TP53* occurred in 2% of cases.Mutations of *TP53* were thereafter investigated in 69 adult B-ALL samples (60 Ph+ and 9 Ph-negative). Since the majority of the studies in leukemia were focused on DNA and resulted in low rate of *TP53* mutations, we aimed to identify RNA mutations and aberrant isoforms. To this purpose three overlapping shorter amplicons covering the entire coding cDNA sequence (GenBank NM_000546.4) and the untranslated exon 1 [amplicon 1 (491 bp): exons 1-5; amplicon 2 (482 bp): exons 5-8; amplicon 3 (498 bp): exons 8-11)] and a longer amplicon (1,317 bp)

starting from exon 1 and ending to exon 11 were sequenced by Sanger method. *TP53* mutations were detected in 7 cases (10.1%), suggesting that these alterations are apparently rare events in B-ALL. They included 4 missense substitutions in the DNA binding and carboxyl-terminal tetramerization domains: C135Y (ex 5), A234T (ex 7), R290C (ex 8) and A347T (ex 10). Interestingly, in two cases aberrant transcripts were identified: 1) a *TP53* isoform characterized by retention of introns 5-6-7 and predicted to encode for a truncated protein due a premature stop codon; 2) a *TP53* isoform in which the DNA binding domain is lost due to an exon conjunction between the exon 4 and the 3' untraslated region (UTR)(ex4-3'UTR: 7579533-7572842, GRCh37/hg19).Next, in order to investigate if low rate of mutations were detectable, we also analyzed whole transcriptome data obtained using next generation sequencing technology (Illumina/Solexa Genome Analyzer) in 3 Ph+ ALL patients. All patients harbored clones ranging from 45% to 94% with *TP53* mutations in the DNA binding and tetramerization domains: C182W (ex 5), T231A (ex 7), L330R (ex 9) in the first patient and Stop394S, D393V/H and G389Y/V (ex 11) in the second one. Moreover, in the first and third patient we detected 10 and 13 base exchanges, respectively, located in intron 6 within 7578166-7578142 region, suggesting a retention of the intron 6 in the primary transcript and the dysfunction of the DNA-binding domain. In conclusion, we demonstrate for the first time that *TP53* alterations at the RNA level (missense substitutions, aberrant exon junctions and intron retentions) are highly frequent in adult B-ALL patients and that testing for *TP53* mutations with a sensitive assay based on RNA analysis is required.Supported by ELN, AIL, AIRC, Fondazione Del Monte Bologna e Ravenna, FIRB 2006, Ateneo RFO grants, PIO program, Programma di Ricerca Regione-Università 2007-2009.

0007

ROLE OF MIR-29A IN THE MOLECULAR PATHOPHYSIOLOGY OF ALL: SYSTEMATIC IDENTIFICATION OF TARGET TRANSCRIPTS

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Background. Several microRNAs (miRs) have been implicated in leukemogenesis, since they play important roles in the control of self-renewal and differentiation, by controlling, the translation or degradation of its target. Recent studies revealed miR-29a as an important player in the regulation of hematopoietic (HSC) and leukemic stem cells (LSC). For instance, reduced levels of miR-29a have been associated with more aggressive disease and worst prognosis in chronic lymphocytic leukemia, mantle cell lymphoma and acute myeloid leukemia (AML). A preliminary database analysis revealed that miR-29a levels in Jurkat (JKT) cells and in acute lymphoblastic leukemias (ALL) are extremely reduced (as compared to normal T-cells). Intriguingly, overexpression of miR-29 in mouse B-cells or HSC results, respectively, in the development of leukemia with B-CLL characteristics or, AML, by converting myeloid progenitors into self-renewing LSC. **Aims.** To explore the role of miR-29a in the molecular pathophysiology of ALL, by carrying a systematic identification of its target transcripts. **Methods.** Synthetic pre-miR-29a, inhibitory anti-miR-29a and respective control molecules were independently electroporated into JKT and 48h post-transfection gene expression profile was obtained using Agilent microarrays. Transcripts simultaneously down-regulated by the pre-miR and up-regulated by the corresponding anti-miR, were compared to the set of predicted targets to identify confident targets. To identify pathways and biological processes modulated by the miR-29a, we used a Functional Annotation Tool (DAVID) and selected targets were validated using qRT-PCR. The functional effect of restoring miR-29a in JKT was evaluated by flow cytometry for apoptosis using annexin V and propidium iodide (PI). Finally, flow-cytometry was used to evaluate the global levels of chromatin 5-hmC and 5mC in Jurkat cells, after pre-miR and anti-miR transfections. **Results.** Among pathways with a statistically significant enriched number of miR-29a target transcripts, we identified: Apoptosis (FAS, BIRC2), WNT (WNT8B/16, FZD4/10, LRP6, TCF7L1), TGF-beta (TGFB3, ACVR2A **BMP8A, SMAD2, BMPR1** LEFTY2,), Jak/Stat (LIF, LIFR, SPRY1) and cancer (MDM2, APC, NRAS, PTEN, PTENP1, RARB, FOS). Further inspection revealed central components of active demethylation (including TET1/2/3 and TDG) and maintenance of DNA methylation following cell division (DMNT3b). qRT-PCR confirmed the significant down-regulation of TET1/2/3 and DMNT3b after pre-miR transfection (pValue<= 0.05, n=3). Functional analysis revealed an increase in the levels of spontaneous apoptosis (Fold=3) and of hydroxy-methylation (34,9% to 59,2% of 5-hmC) in JKT cells, after treatment with pre-miR and anti-miR, respectively. **Summary/ Conclusions.** Both, oncogene hypomethylation or tumor suppressor hypermethylation can lead to oncogenic transformation. For instance, mutations in the DNMT3A (leading to hypomethylation) or the TET family (leading to hypermethylation) are found in a significant fraction of myeloid disorders. We have identified several target transcripts of miR-29a that are associated with pro-oncogenic pathways. Moreover, we show that increased levels of miR-29a leads to increased apoptosis in Jurkat cells. Taken together, reduced miR-29a levels found in ALL may contribute to the molecular pathophysiology of the disease; trough the consequent increase in the level of its target transcripts, which include central components involved in the epigenetic control of transcription by *de novo* methylation or active demetylation. **Support**. FAPESP, CNPq. luhabib@yahoo.com.br

0008

OVARIAN INFILTRATION IS CELL LINE SPECIFIC AND ASSOCIATED WITH HIGH CXCR4 EXPRESSION IN A XENOGRAFT MODEL OF PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background. Acute lymphoblastic leukemia (ALL) is the commonest hematological malignancy in children. With improved treatment a high cure rate has been achieved, however management of relapsed disease and reduction of treatment-related toxicity remain major challenges. Although ovarian involvement is commonly detected at post-mortem in female patients with relapsed disease, little is known about the mechanisms underlying leukemic infiltration of this tissue. As well as being of interest to the pathogenesis of relapse, understanding ovarian involvement also has implications for fertility preservation such as the use of cryopreserved ovarian tissue for post remission re-implantation in girls with previous ALL.We hypothesize that leukemic cell dissemination to extramedullary sites such as the ovaries is associated with the abnormal expression of chemokine receptors which play important roles in governing physiological leukocyte trafficking as well as in metastasis of many types of cancer. To test this we have developed a xenograft model of extramedullary ALL using human leukemic cell lines and immunodeficient (NOD/SCID/IL2R gamma null) mice. Using this model we have identified two Philadelphia positive pre-B ALL cell lines which differ in their capacity to home to the ovaries with one producing striking ovarian infiltration (SupB15) and the other (SD1) showing no signs of ovarian involvement but producing aggressive disease elsewhere. **Aims.** To identify chemokine receptors associated with homing of acute lymphoblastic leukemia to the ovaries. **Methods.** Initial screening was performed by quantitative PCR for 17 candidate chemokine receptors in the ovarian homing (SupB15) and non-homing (SD1) cell line. Promising candidates were further evaluated by flow cytometry and functional assays. Expression of the corresponding chemokine ligands in murine ovarian tissue was assessed by qPCR. **Results.** Only two chemokine receptors were up-regulated in SupB15 cells compared to SD1 cells; CXCR4 and CCR9. High levels of CXCR4 expression by SupB15 were confirmed by FACS and immunofluorescence. In addition SupB15 cells were shown to bind the CXCR4 ligand CXCL12 using fluorescently labelled chemokine and showed efficient chemotaxis towards CXCL12 in transmigration assays. Migration could be inhibited by use of the CXCR4 antagonist AMD3100. In contrast SD1 cells showed lower levels of CXCR4 expression by FACS and no directional migration to CXCL12. We could not confirm CCR9 surface expression by FACS on SupB15 cells or transmigration in response to its ligand CCL25. This data is further supported by qPCR analysis of chemokine ligand expression by murine ovaries which show high levels of CXCL12 and only very low levels of CCL25 in resting tissue. Further work will investigate the effect of CXCR4-inhibition on the ability of SupB15 to infiltrate the ovaries *in vivo*. **Summary.** Despite both carrying the t(9:22) translocation these two cell lines produce distinct patterns of extramedullary spread with a striking difference in their ability to infiltrate the ovaries. Here we provide evidence that the chemokine receptor CXCR4 and its ligand CXCL12 may be important in determining spread to this important sanctuary site.

0009

GENE EXPRESSION-BASED IN SILICO SCREENING FOR GLUCOCORTICOID-SENSITIZING THERAPEUTICS IN MLL-REARRANGED INFANT ALL IDENTIFIES PI3K INHIBITORS AS POTENTIAL MODULATORS OF PREDNISOLONE RESISTANCE

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Background. Obtaining successful treatment results for *MLL*-rearranged Acute Lymphoblastic Leukemia (ALL) remains challenging, to some extent due to poor glucocorticoid (e.g. prednisone and dexamethasone) responses. Thus, overcoming resistance to these drugs may be a crucial step towards improving prognosis. However, despite intensive research, little is known about the

mechanisms of resistance. **Aims.** Therefore we aimed to produce a gene expression signature associated with *in vitro* prednisolone resistance in *MLL*-rearranged infant ALL, and identify therapeutic agents that reverse the profile and induce prednisolone sensitivity. **Methods.** By comparing gene expression profiles (Affymetrix HU133plus3.0 GeneChips) from *MLL*-rearranged infant ALL patients either sensitive or resistant to prednisolone *in vitro*, we defined a gene signature associated with prednisolone resistance. Using this signature, we applied Connectivity Map analysis to perform an *in silico* screen for agents potentially capable of reversing the prednisolone-resistance profile and induce sensitivity. **Results.** Connectivity map analyses revealed that LY294002, a PI3K inhibitor, would potentially fulfill this task. Subsequent validation experiments demonstrated that indeed LY294002, as well as other known PI3K inhibitors, markedly sensitized otherwise resistant *MLL*-rearranged ALL cell to prednisolone *in vitro*. Using quantitative real-time PCR analyses, we validated the modulating effects of LY294002 on the expression of the genes present in our prednisolone-resistance profile. This showed that the prednisolone-sensitizing actions of LY294002 are indeed mediated by inhibition of PI3K, as one of the genes down-regulated in response to LY294002, *i.e. PARVB*, represents a known PI3K target. **Conclusions.** We conclude that implementing FDA-approved PI3K inhibitors in current treatments may potentially improve the GC-response, as well as the prognosis, for patients with *MLL*-rearranged ALL.

0010

FREQUENCIES AND PROGNOSTIC IMPACT OF RAS MUTATIONS IN MLL-REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA IN INFANTS

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Background. Acute Lymphoblastic Leukemia (ALL) in infants represent an aggressive malignancy associated with a high incidence (~80%) of *MLL* translocations like t(4;11) generating MLL-AF4 fusion proteins. Recent attempts to mimic MLL fusion-driven leukemogenesis in mice raised the question whether or not these MLL fusions require secondary hits to induce leukemia. Based on previous reports, *RAS* mutations may represent likely candidates, but results are inconclusive and contradicting on the incidence of these mutations in *MLL*-rearranged ALL. **Aims.** Therefore, our aim was to elucidate the precise frequencies and the potential role (in terms of disease aggressiveness and clinical outcome) of *RAS* mutations in *MLL*-rearranged infant ALL patients, and their relation with known (e.g. WBC, age, and glucocorticoid response) and putative (e.g. *HOXA* expression) prognostic factors.

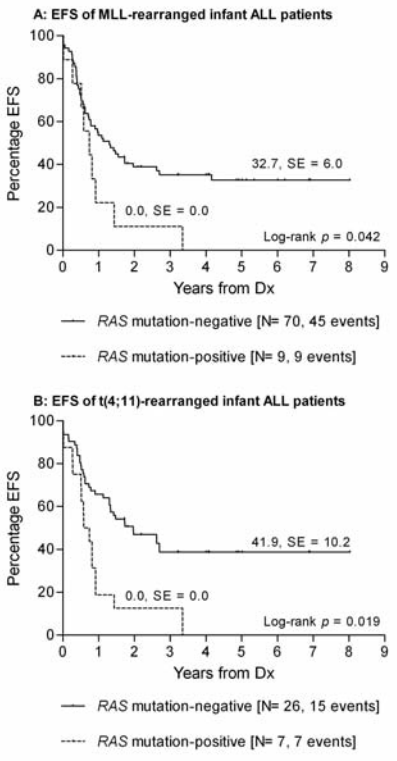

Figure 1. Survival of RAS mutated and non-mutated infants .

Methods. For this, PCR and sequence analysis were used to screen a large cohort (>100 samples) of infant ALL cases for the presence of *RAS* mutations, and studied our results in relation with various clinical parameters and prognostic impact. **Results.** *RAS* mutations were detected in ~14% of all cases tested, with a significantly higher frequency of ~24% in t(4;11)-positive patients (p=0.04). The presence of a *RAS* mutation was associated with a poor outcome in both the *MLL*-rearranged infant ALL patients, as well as in t(4;11)-positive cases alone (Figure 1). The 5-year EFS rates for the *RAS* mutation-positive and negative *MLL*-rearranged cases was 0· 0±0· 0 % vs. 32.7±6.0%(p=0.042). Furthermore, we identified the presence of a *RAS* mutation as an independent predictor (p=0.019) for poor outcome in *MLL*-rearranged infant ALL, with a hazard ratio of 3.194 (95% confidence interval: 1.211-8.429). Also, *RAS*-mutated patients appeared to have significant higher white blood cell counts at diagnosis (p=0.013), and are significantly more resistant to glucocorticoids *in vitro* (p<0.05). Finally, we demonstrate that *RAS* mutations, but not the lack of *HOXA9* expression nor the expression of *AF4-MLL* are associated with poor outcome in t(4;11)-rearranged infant ALL. **Summary and Conclusions. .** We conclude that *RAS* mutations represent genuine secondary hits in *MLL*-rearranged infant ALL and are an independent predictor for clinical poor outcome in this already unfavorable type of childhood leukemia. Moreover, we propose future risk-stratification based on abnormal *RAS*-pathway activation and that *RAS*-mutated infants could benefit from additional treatment with *RAS*-pathway inhibitors.

0011

TAGONES, MINOR MATURE NK AND T LYMPHOCYTE SUBPOPULATIONS INTERFERING WITH MRD T-ALL ANALYSIS - A NOVEL STRATEGY PROPOSED

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Background. In Acute T Lymphoblastic Leukema (T-ALL), minimal residual disease identification by multiparameter flow cytometry, has been based on simultaneous assessment of cytoplasmic and surface CD3 antigen along with combinations of CD7 and CD45 common connecting antigens. In the individual protocols there are used specific antigens that mainly characterize immature epitopes like Tdt, CD99, CD34, CD117, CD10, CD1a etc. Recently however ı Roshal et al, Cytometry B Clin Cytom. 2010 May;78(3):139-46, showed that immediately post treatment those antigens are significantly down modulated, providing false negative results in MRD identification based on these immature antigens. In contrast mature T lymphocyte antigens like CD7, CD2, CD4, CD8, CD5, TCRab, TCRgd, appear to retain their expression during therapy. AIM The aim of this study was to evaluate a novel gating strategy based on mature antigens, initially using as a control cohort, samples of non-T ALL receiving therapy. **Methods.** We analyzed 25 non T-ALL marrow samples from patients recovering from therapy (B-ALL, AML, Lymphomas) as well as 12 normal blood and reactive marrows. **Results.** We located minor mature NK and T cell subpopulations interfering in presumed T-ALL empty spaces. 1) CD45 bright NK cells CD16+ or CD56+ with cytoplasmic CD3 epsilon expression, CD7+, CD2, CD5-, TCR- and surface CD3 negative 2) CD45 bright T4 and T8 lymphocytes with down regulated surface CD3 and TCRab expression, CD2+, CD5+. 3) NK2 CD56bright lymphocytes with reduced CD45 intensity. All the above populations were consistently found in regenerating and normal condition in minus log3 and log4 concentrations and interfered with our MRD T-ALL analysis. In comparison to hematogones in B-ALL they were named for simplicity tagones. The novel strategy of MRD analysis was planned with five color combinations using as common backbone antigens, surface and cytoplasmic CD3, CD7, CD16 and CD45, while tested antigens were all mature (CD2, CD4, CD5, CD8, TCRαβ, TCRγδ, CD56). Within this analysis platform, tagone patterns were consistently similar between non-T-ALL subjects. We then prospectively applied this strategy in the evaluation of T-ALL MRD patterns (n=18). MRD was considered positive, when there were noted deviations from the tagones pattern of expression. They were quantitated up to -log4 concentration. **Conclusions.** We provide a feasible strategy of multiparameter MRD detection in T-ALL based on the concept of discrimination from the patterns normal minor populations of mature T and NK cells, imitating expressions of antigens found in T-ALL

0012

TP53 MUTATIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) ARE RELATIVELY FREQUENT IN MOLECULARLY NEGATIVE CASES OF BOTH B- AND T-LINEAGE AND CORRELATE WITH POOR RESPONSE TO INDUCTION THERAPY

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Background. *TP53* mutations occur in several cancers including hematologic malignancies, particularly chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML), where the incidence is of 5-10% and 7%, respectively. While in these neoplasms *TP53* mutations have been widely investigated and correlate with a poor prognosis, their incidence and role in acute lymphoblastic leukemia (ALL) remain controversial. **Aims.** To investigate the incidence of *TP53* mutations and their correlation with known molecular aberrations and outcome, we analyzed a cohort of 98 newly diagnosed adults with ALL. In 10 cases, we also analyzed paired relapse samples. **Methods.** Genomic DNA was extracted from mononuclear cells of patients enrolled in different GIMEMA protocols. All samples contained at least 70% of leukemic blasts. In 10 cases paired diagnosis-relapse material was analyzed. Sixty-two patients had a B-lineage ALL (B-ALL) and 36 a T-ALL. Molecular screening showed that, within B-ALL cases, 25 harbored the *BCR/ABL*, 9 the *ALL1/AF4* and 4 the *E2A/PBX* transcripts. The remaining patients were negative for the presence of the aforementioned fusion genes. Within T-ALL, 4 cases harbored the *SIL/TAL1, 1 NUP98/RAP1GDS1* and *1 SET/NUP214* rearrangement.*TP53* mutation analysis was performed using a microarray-based sequencing assay, the AmpliChip p53 Research Test (Roche Molecular Systems), which allows sequencing of the entire coding region of the *TP53* gene, including exons 2-11 splice sites. Results were validated by direct sequencing. **Results.** The AmpliChip p53 assay identified *TP53* missense mutations in 8 patients (8.16%) studied at diagnosis. Within the mutated patients, 4 had a B-ALL (6.4%): 3 cases (12.5%) had no known molecular abnormality and 1 harbored the *BCR/ABL* rearrangement (4%). No mutations were found in *E2A/PBX1+* and *ALL1/AF4+* cases. Within the T-ALL group, 4/36 carried *TP53* mutations (11.1%): none of these cases harbored known molecular aberrations. Overall, *TP53* mutations were found in 13% of ALL cases without known molecular abnormalities.Sanger sequencing confirmed all the mutations but one, suggesting the presence of a sub-clonal population. Furthermore, of the 10 paired patients studied, 2 acquired the mutation at relapse (20%): one patient had a *BCR/ABL+* B-ALL and the other a T-ALL. Patients harboring *TP53* mutations were predominantly males (M/F= 6/2) and were younger than *TP53* wild-type (wt) cases (*TP53* mut 30 *vs TP53* wt 36.5 years).Since the majority of the mutations occurred in molecularly negative patients, response to induction was evaluated in this subset: of the 47 evaluable patients, 12 were refractory to induction and, remarkably, 25% were *TP53* mutated. **Conclusions.** These results indicate that *TP53* mutations frequency in ALL at the onset of the disease is similar to that reported in CLL and AML. Furthermore, they indicate that, within patients without known molecular aberrations of both T- and B-lineage, *TP53* alterations can be detected in 13% of cases and therefore could be particularly useful for the prognostic stratification of these subgroups. Indeed, although statistical significance was not reached because of the small number of *TP53* mutated patients, our results indicate that *TP53* mutations have a deep impact on response to induction treatment and achievement of complete remission.

0013

SRC KINASE INDUCED PHOSPHORYLATION OF ANNEXIN A2 MEDIATES GLUCOCORTICOID RESISTANCE IN MLL-REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Glucocorticoids (e.g. prednisone and dexamethasone) are commonly used in the treatment of childhood acute lymphoblastic leukemia (ALL). Unfortunately, infants with ALL (<1 year of age) characterized by translocations of the *MLL* gene are frequently resistant to these drugs. As poor glucocorticoid responses are firmly associated with therapy failure, overcoming glucocorticoid resistance may be a crucial step towards improving prognosis. In search of insights into glucocorticoid resistance mechanisms, we generated a gene expression signature associated with *in vitro* prednisolone resistance in *MLL*-rearranged infant ALL. One of the genes strongly discriminating between resistant and sensitive patients appeared to be *ANXA2*, encoding human annexin A2. **Aims.** Here we aimed to functionally study the role of annexin A2 in prednisolone-resistant *MLL*-rearranged ALL. **Methods.** *ANXA2* expression was validated by quantitative RT-PCR and immunoblotting in *MLL*-rearranged cells. To study the role of annexin A2, Src kinases or p11 in prednisolone resistance, we performed RNAi experiments using lentiviral constructs expressing either a non-targeting shRNA or shRNAs directed against *ANXA2*, Src kinases or *S100A10*. *In vitro* prednisolone response was determined by MTT assays. **Results.** *MLL*-rearranged cells resistant to prednisolone *in vitro* expressed elevated levels of annexin A2 and its phosphorylated form (active). Further investigation demonstrated that the underlying factor of this association was the presence of Src kinase induced phosphorylation (activation) of Annexin A2, a process requiring the adaptor protein p11 (encoded by *S100A10*). shRNA-mediated knock-down of annexin A2, FYN, LCK, or p11, all led to inhibition of annexin A2 phosphorylation and resulted in marked prednisolone sensitization in otherwise resistant *MLL*-rearranged ALL cells. Likewise, exposure of prednisolone-resistant *MLL*-rearranged ALL cells to various known Src kinase inhibitors exerting high specificity towardsFYN and/or LCK had similar effects. **Conclusions.** In conclusion, we here present a novel mechanism of prednisolone resistance in *MLL*-rearranged ALL, and propose that inhibition of annexin A2 phosphorylation embodies an attractive therapeutic strategy for overcoming resistance to glucocorticoids in this highly aggressive type of leukemia.

0014

THE POTENTIAL OF AURORA KINASE B AS A THERAPEUTIC TARGET IN CHILDHOOD ACUTE LEUKEMIA

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Background. Aurora kinases (AURK) A and B are known regulators of mitosis and are overexpressed in a large number of human cancers, including leukemia. Several AURK-inhibitors have shown anti-tumor activity *in vitro* and *in vivo*. However, the efficacy of AURK inhibition in the treatment of childhood acute leukemia is largely unexplored. **Aims.** We investigated the effect of inhibiting AURKA and AURKB in leukemic cells of children with newly diagnosed acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), to determine the potential of these two kinases as new targets for therapy. **Methods.** Affymetrix gene expression data of 297 ALL, 237 AML and 8 normal bone marrow (nBM) samples were analyzed for AURKA and B mRNA expression levels. Protein expression levels in 172 pediatric ALL and 10 nBM samples were determined with a reverse phase protein array. Functional studies were performed in ALL and AML cell lines, in which AURKA and B were silenced using a short hairpin RNA with a lentiviral delivery system or LNA-containing oligonucleotides. Sensitivity of leukemic cell lines and primary patient samples to the AURKB-selective inhibitor Barasertib-HQPA (AZD1152-HQPA) was tested *in vitro* with an MTS assay. **Results.** AURKA and B mRNA levels were low in ALL and AML patients. In contrast, Aurora A and B proteins were expressed to a greater extent in patients ($p<0.0002$), especially in ALL cases with an E2A-PBX1 translocation ($p<0.002$), than in nBM mononuclear cells. Silencing of AURKA by shRNA and by LNA-oligonucleotide caused no or only minor growth delay in several cell lines reflecting genetic subtypes typically found in pediatric

ALL and AML. In contrast, silencing of AURKB resulted in proliferation arrest and apoptosis in these cells. Furthermore, 18 out of 20 ALL and AML cell lines tested were highly sensitive to the AURKB-selective inhibitor Barasertib-HQPA in the nanomolar range (IC50 = 19-233 nM), and differential sensitivity was observed between primary patient samples. **Summary and Conclusions. .** In adult AML, clear responses are observed with Barasertib in early clinical trials. In agreement, we show that inhibition of Aurora B by shRNA, LNA-based mRNA antagonist and Barasertib-HQPA has an anti-proliferative and pro-apoptotic effect on childhood acute leukemia cells, whereas targeting Aurora A did not have a major effect. Aurora B could therefore be a promising new target for therapy in the treatment of pediatric ALL and AML.

0015

GENE EXPRESSION PROFILING, HIGH-DENSITY GENOME-WIDE GENOTYPING AND COPY NUMBER ANALYSES OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background. Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by a clonal proliferation of malignant lymphoblasts, which is characterized by accumulation of genetic alterations in B or T lymphoid precursor cells. ALL is the most common leukemia in children, but is a rare disease in adults. In recent years this disease has been extensively studied in children using various genome-wide molecular approaches, however, studies in adults are lacking. **Aims.** We have analyzed a cohort of 71 paired, i.e. diagnosis and remission, adult ALL cases using gene expression profiling with Affymetrix HGU133 Plus 2.0 Genechips and genome-wide genotyping and copy number analyses using Affymetrix 6.0 DNA Mapping GeneChips. These high-resolution SNP-arrays enable the determination of recurrent focal, possibly cryptic, genetic lesions. **Methods.** In paired diagnosis and remission samples genotype calls per SNP were determined using the Birdseed algorithm and copy numbers per SNP using dChip. The ALL cohort included 53 B-ALL cases and 18 T-ALL cases, which were all extensively molecularly characterized, i.e., all known recurrent ALL specific molecular aberrations were determined. Unsupervised analyses of the ALL cases based on gene expression profiling data demonstrated that besides the separation of B-ALL and T-ALL, various molecularly-defined ALL subsets carried distinct gene expression profiles. **Results.** Genome-wide genotyping demonstrated (1) recurrent aberrations involving *CDKN2A/B* (65%), *IKZF1* (29%), *PAX5* (29%), BTG1 (23%) and *RB1* (7%) at higher frequencies than previously shown by others, (2) that all T-ALL cases carried a copy number change involving the CDKN2Apathway (two cases that did not carry an allelic loss of *CDKN* had lesions in *CDKN2A-IP* or *CDKN2A-IPNL*), (3) additional recurrent aberrations in *MKKS* (9%), resulting in significant down regulation of gene expression, and *KRAS* (7%), (4) selected cases with unbalanced translocations involving *BCR-ABL*, *NUP214-SET*, *NUP214-SQSTM1* and *TCF3-HLF*, as well as illegitimate V(D)J recombination involving *CRLF2* and *STIL*, (5) genetic aberrations involving *SUZ12*, i.e., a core component of the Polycomb Repressive Complex 2 (PRC2), in 3 T-ALL cases. This ensued sequencing of the *SUZ12* gene, i.e., the region encoding the Zn-finger and VEFS-box (EZH2 interaction domain) in 200 ALL cases, resulting in the identification of missense or nonsense *SUZ12* mutations in 3 T-ALL cases. **Conclusions.** We have demonstrated that high-quality/high-resolution SNP-arrays grants the ability to determine submicroscopic genetic lesions and unbalanced translocations. Additionally, we have shown that many known recurrent ALL-specific copy number variants are found at a higher frequency than previously reported. Finally, we have also identified novel aberrations, such as those in the *SUZ12* gene, which are currently subjected to mutational analyses.

Acute lymphoblastic leukemia - Clinical

0016

IMPACT OF MINIMAL RESIDUAL DISEASE LEVEL DURING THE FIRST-LINE IMATINIB THERAPY ON LONG-TERM OUTCOMES OF ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULTS WITH PH-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The combination of imatinib with chemotherapy as a front-line treatment has demonstrated an improved complete response rate and an increased applicability of allogeneic stem cell transplantation (SCT), thus allowing a better short-term outcome in adults withPhiladelphia chromosome-positive acute lymphoblastic leukemia (Ph-positive ALL). However, with prolonged follow-up, a substantial proportion of transplants and practically almost all non-transplants continue to die as a result of relapse. Most published studies in the imatinib era include quantitative minimal residual disease (MRD) findings, but the long-term outcome in relation to molecular response to any given therapeutic approach remains to be determined in Ph-positive ALL. **Aims.** Here, with a more sizable patient population and a longer follow-up, we conducted a systemic re-evaluation to identify risk factors that affect long-term transplantation outcome in the imatinib era, and focused particularly on the prognostic relevance of MRD level at each pre-SCT treatment stage. **Methods.** Between September 2000 and December 2009, 95 adults [median age, 34 years (range, 15 to 59 years)] with newly diagnosed Ph-positive ALL who received a uniform treatment protocol of allogeneic SCT following imatinib (400 mg/d for 4 weeks at each cycle) plus conventional chemotherapy (alternating modified hyper-CVAD and high-dose cytarabine/mitoxantrone) were included in this analysis. All patients were transplanted from a fully matched sibling or a suitably matched (≤2-allele mismatched) unrelated donor after the completion of the second imatinib cycle. MRD monitoring for BCR-ABL1 transcript is centrally evaluated by real-time quantitative PCR (4.5 log sensitivity) through handling of bone marrow samples from all patients (Research Institute of Molecular Genetics, The Catholic University of Korea,Seoul,Korea). All patients in the study provided written informed consent, and the study protocol was approved by the institutional review board of The Catholic University of Korea. **Results.** After the first imatinib cycle, 33 of the 95 patients (34.7%) achieved at least major molecular response [MMR; including 12 complete molecular response ($CMR^{4.5}$)]. Frequencies of achieving a reduction in BCR-ABL1 transcript levels of 1% to <0.1% and <1% at this stage were 27 (28.4%) and 35 (36.8%), respectively. After the second imatinib cycle, 68 patients (71.6%) achieved at least MMR (including 27 $CMR^{4.5}$), and the remaining 27 patients showed an insufficient molecular response [1% to <0.1% (n=9; 9.5%) and <1% (n=18; 18.9%), respectively]. Eighty-eight (92.6%) of the 95 patients received SCT in first complete response. After a median follow-up of 60 months, the 5-year cumulative incidence of relapse and nonrelapse mortality were 24.9% and 18.8%, respectively, and the 5-year disease-free survival and overall survival rates were 61.5% and 65.8%, respectively. The most powerful predictive factor affecting relapse, disease-free survival, and overall survival was the achievement of good quality of molecular response (MMR or $CMR^{4.5}$) after the second imatinib cycle ($P<0.001$). **Summary and Conclusions. .** In the era of tyrosine kinase inhibitors, prospective assessment of the extent of MRD reduction may allow us to identify subgroups of Ph-positive ALL transplants at high risk of relapse and provide potential guidelines for the development of new risk-adapted, MRD-based therapeutic approaches.

0017

IMPROVED RISK-STRATIFICATION AND OUTCOME PREDICTION IN CHILDREN WITH AVERAGE RISK 1 PRECURSOR-B ACUTE LYMPHOBLASTIC LEUKEMIA USING A 19-MICRORNA SIGNATURE

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Background. Risk stratification has led to a tremendous improvement of the 5-year overall survival rates in childhood acute lymphoblastic leukemia (ALL). The average risk group 1 (AR1), consisting of all non-very low risk and non-very high risk B-cell lineage ALL patients, is the largest patient group accounting for 57% in our experience. Despite the good overall survival, the total number of relapses observed in this AR1 group is considerable. **Aims.** In this study, we aimed at identifying a marker able to predict relapse, at the stage of diagnosis. To this end, we focused on the recently discovered microRNAs of which their expression has been reported to hold prognostic power in other cancer types. **Methods.** A total of 693 microRNAs were profiled using automated high-throughput quantitative stem-loop RT-PCR in a cohort of diagnostic bone marrow samples from AR1 pediatric precursor B-cell ALL patients in continuous complete remission (follow-up>6 years) and patients who experienced relapse. All patients were treated according to the EORTC-CLG protocol 58951 (December 1998-August 2008). The ethical committee approved the study and informed consent was obtained from the patients and/or their parents. Statistics were performed using SPSS17 and R (Bioconductor). **Results.** Logistic regression analysis and Prediction Analysis of Microarray allowed us to identify a 19-microRNA signature, prognostic for relapse within this group. The signature has an accuracy, sensitivity and specificity of 77%, 69% and 84%, respectively. Currently, the signature is evaluated in an independent validation cohort. Notably, several of the microRNAs present in this signature are known oncogenes or tumor suppressor genes. Multivariate analysis, including the microRNA signature, white blood cell count and age, shows that the 19-microRNA signature is an independent prognostic factor. **Conclusions.** The identified 19-microRNA signature is a unique and powerful tool for further risk-stratification. The method and signature are suitable for routine laboratory testing and will be further evaluated in a prospective study. **Correspondence to:** farzaneh.ghazavi@ugent.be

0018

PH+ ALL IN THE ELDERLY IS NOT ASSOCIATED WITH POORER PROGNOSIS THAN PH-NEGATIVE ALL IN THE TKI ERA. COMPARISON OF TWO PROSPECTIVE PARALLEL TRIALS

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Background and Aims. Among elderly patients with ALL, those with Ph+ had historically a poorer prognosis. Excellent short-term results have been obtained with TKI inhibitors together with minimal or moderate amount of chemotherapy, but a continuous pattern of relapses has been observed over time. A comparison of two prospective parallel trials conducted by the Spanish PETHEMA Group in elderly patients with Ph+ or Ph- ALL is herein performed. **Patients and Methods.** Patients older than 55 yr. were included in the ALLOLD07 and ALL OPH07 trials if they have Ph- or Ph+ ALL, respectively. ALLOLD07 trial (derived from the Elderly ALL trial from the EWALL Group [Göckbuget et al, Blood 2008; 112: Abstract 304]) included induction with vincristine (VCR) dexamethasone (DXM) and idarubicin (IDA), in phase 1, and cyclophosphamide (CPM) + cytarabine (ARA-C) in the Phase 2. Consolidation included 6 alternating cycles with intermediate-dose methotrexate (MTX)+asparaginase (ASP) (odd cycles) and ARA-C (even cycles). Maintenance therapy included merpatopurine (MP)+MTX up to 2 yr, with monthly reinduction cycles with VCR and DXM during the first year. ALL OPH07 tiral included induction with imatinib, VCR and DXM and maintenance with imatinib+MP+MTX up to 2-yr, with monthly reinduction cycles with VCR and DXM during the first year. Imatinib therapy was given during the third year. **Results.** Between July 2007 and December 2011, 28 valid patients were included in the ALLOLD07 and32 in the ALLOPH07 trial. Both groups of patients were comparable for the main clinical and biologic characteristics of ALL. Early death was observed in 3(11%) of patients in the ALLOLD07 trial vs. 4 (13%) in ALL OPH07, failure occurred in4 pts (14%) vs. 1(3%), and CR was attained in 21(84%) vs. 26(96%), respectively. With a median follow-up of 17 (1-52) months in both protocols, 3 patients withdrawn from the ALLOLD07 (vs. none in the ALLOPH07), 10 (48%) relapsed (vs. 9[35%]) and 1(5%) (vs. 2 [8%]) died due to treatment-related mortality. In all, 7 patients (25%) (vs 15 [47%]) are alive in first CR.The medians (95%CI) of CR duration were 27(13-41) months in ALLOLD07 trial vs. 37(13-43) in the ALLOPH07, and the medians of OS were 16(8-23) months (vs. 22[9-35]), without significant differences between the two protocols. The duration of neutropenia in induction was significantly longer in the ALLOLD07 trial, as was extramedullary grade III-IV toxicity, especially infections. No differences in the post-induction toxicity were observed on comparison of both protocols. **Conclusions.** From the data of these two parallel protocols, Ph+ ALL in the elderly is not associated with poorer prognosis in the TKI era. However, improvement in the therapy of both Ph+ and Ph- ALL in elderly patients is clearly needed.Supported by grants RD06/0020/1056 from RTICC, PI10/01417 from FIS, Instituto Carlos III, and P-EF-11 from Jose Carreras Leukemia Foundation.

0019

BONE MARROW CLEARANCE OF LEUKEMIC BLASTS ON DAY 14 OF INDUCTION DETERMINES PROGNOSIS AND HELPS IN STRATIFICATION OF ADOLESCENTS AND ADULTS WITH *de novo* ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. early clearance of leukemic blasts after induction had been shown to correlate with prognosis and to help in stratifying ALL therapy. Timing and methodology as well as methods of quantification of residual leukemic blasts varied significantly. **Aims.** to assess the impact of clearance of marrow leukemic blasts on day 14 of induction on prognosis and its value in stratifying *de novo* ALL in adolescents and adults. **Patients and Methods.** 163 (53 female, 110 male) consecutive patients were treated between 2003-2011 with a unified CALGB-based induction (Adriamycin 50mg/m^2 d1-3, Cyclophsphamide 1.5gm/m^2 d1, vincristine 1.4/m^2 d1,8,15,22, Prednisone d1-28 , L-aspaaraginase) followed by HIDAC consolidation. Cytomorphology and flow-cytometry of bone marrow aspirates and biopsies (BMA) was reported as BMA- if blasts <5% and BMA+ if ≥5%. Those with high risk features [high WBC count (>100K in T- or >30K in PreB-), CNS+, high risk cytogenetics (Ph+) or >4 weeks to achieve remission or BMA+.] were offered Allo-HSCT if available. Salvage was tried in BMA+. Day 28 BM biopsy was done to assess complete remission (CR) status. Of the 163 patients, 72% were PreB- vs. 28% T-ALL. Median age was 21-y (14-63). Median follow up of survivor was 21 mo(0.68-96.5). **Results.** 134 (82%) were BMA -ve and 29 (18%) BMA+ with no difference in phenotype (p 0.65), high WBC count (p 0.95), age (p.95). BMA+ had more Ph+ (28% vs. 10%;p=0.015), CNS (28% vs. 10%;p=0.03) and a trend of male dominance (79% vs. 65%; p= 0.06). 50% of BMA- carried high risk features while all (100%) BMA+ were considered high risk. Of BMA-, 130 (97%) achieved CR1 vs. 16 (57%) of BMA+(p=0.005) and 50% from BMA- received alloHSCT (BMA-BMT+) vs. 54% of BMA+ (BMA+ BMT+)(p 0.95). OS of BMA- was 49.5%+0.05 vs. 20.3%+0.08 for BMA+ (p 0.002; HR=2.2+0.26). OS values of different groups in relation to AlloHSCT (BMT) were: BMA-BMT+ 57% +0.06, BMA-BMT-: 41.4+/-0.07; (p=0.06) and BMA+BMT- of 33.3%+/-0.12 vs. BMA+ BMT- of zero% (P=0.002). CIR for BMA- was 0.56 (95% CI=0.54-0.57) vs. 0.69 (95% CI=0.66-0.72) for BMA+ (P=0.2) with a HR of 1.5 (0.85-2.67; p=0.2) .Using Competing risk regression modeling (CRR) by Fine & Gray, BMT had insignificant lowering of CIR in BMA- (0.61+/-0.005 vs. 0.5 +/- 0.005; p=0.3) but significant in BMA+ (0.86+0.026 vs. 0.47+0.018; p=0.01). BMA- had a trend for better DFS vs. BMA+(0.36+0.04 vs. 0.22+0.08; p=0.1). The impact of alloHSCT in improving DFS was greatly evident in BMA+ (BMT- 0%, BMT+ 0.33+/-0.12; p=0.02) vs. BMA- (BMT- 0.3+/-0.06, BMT+0.42+/-0, 06; p=0.2). In the BMA- , there was no significant impact of other high risk factors including age (p=0.45), WBC (p=0.2), CNS+ (p=0.2), poor cytogenetics (p=0.9) or BMT (p=0.2). **Conclusions.** persistence of ≥5% marrow leukemic blasts on day 14 of induction is a strong prognostic and stratification tool in ALL and is associated poor outcomes. AlloHSCT improves outcome of *de novo* ALL and is the only option for meaningful survival for those with significant residual disease on day 14 of induction.

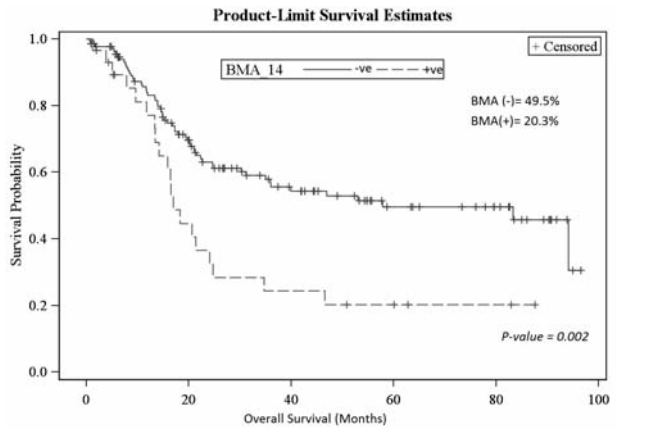

Figure 1.

0020

MINIMAL RESIDUAL DISEASE DETECTION IN CHILDHOOD B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA. MULTICOLOR FLOW CYTOMETRY AND PCR OF FUSION GENE TRANSCRIPTS DATA COMPARISON

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Background. Minimal residual disease (MRD) monitoring by flow cytometry (FC) or polymerase chain reaction (PCR) is a strong tool for risk-adapted treatment in childhood acute lymphoblastic leukemia (ALL). IgH/TCR rearrangements monitoring by real-time quantitative PCR (RQ-PCR) is very laborious and costly approach. Hence other methods - FC and PCR of fusion gene transcripts (FGt) - can be used for long-term MRD monitoring. **Aims.** To evaluate qualitative concordance between MRD data assessed by FC and PCR of FGt in children with ALL. **Methods.** Concurrent detection of MRD by multicolor FC, RQ-PCR and RT-PCR was performed in 331 follow-up bone marrow samples from 77 children. Among them 34 patients (pts) carried *ETV6-RUNX1* FGt, 4 pts - *TCF3-PBX1*, 1 pt - *BCR-ABL*, 3 pts - *SCL-TAL1* while in other 35 pts various types of *MLL*-rearrangements were detected. 29 pts had CD10(-), 45 pts - CD10(+) B-lineage ALL and 3 pts - T-ALL. 115 samples were obtained during remission induction while 216 - during consolidation/intensification. **Results.** Sensitivity of FC MRD detection varied from 0.01% to 0.001%. PCR sensitivity ranged from 5×10^{-5} to 1×10^{-5}. 100 of 331 samples were MRD-negative by both methods, 36 (10.87%) - were negative by FC but positive by RT-PCR, while in 8 samples (2.42%) tumor cells were detected by FC only. Remaining 187 samples were MRD-positive by both techniques. Qualitative concordance of 86.71% between FC and PCR data was found. Concordance in *MLL*-rearranged(88.51%), *ETV6-RUNX1*-positive(88.00%) and *TCF3-PBX1*-positive(91.67%) cases was very similar (p=0.879). Samples with and without normal B-cell precursors (BCP) were analyzed separately, because presence of BCP in follow-up samples is known to be an obstacle for FC data analysis. Qualitative concordance in BCP-negative and BCP-positive samples was very similar (84.21% and 90.53% respectively, p=0.123). In contrast, concordance of FC and PCR data in samples obtained during remission induction and during consolidation/intensification was significantly different (78.26% and 91.20% respectively, p=0.002). As long as FC data analysis in CD10(+) and CD10(-) B-lineage ALL patients bases on different approaches, these types of leukemia were also analyzed separately. High qualitative concordance was found in samples from both B-lineage ALL types (85.37% and 89.81% respectively, p=0.300). Direct comparison of quantitative FC and RQ-PCR data is impossible but MRD values in FC-negative/PCR-positive samples were lower than in samples, where tumor cells were detected by both methods (median 0.011%, range 0.001-0.413% and median 0.534, range 0.001-56.491% respectively, p<0.0001). So FC appears to be better for the quantitative MRD evaluation however FGt detection by PCR is more appropriate for qualitative MRD assessment. Hence, FC is more applicable for MRD monitoring during early treatment phases, when the precise MRD value is essential while PCR for FGt is more useful for later time-points when any MRD-positivity corresponds to poor outcome of childhood ALL. **Conclusions.** Qualitative concordance between PCR-based FGt detection and FC data achieved 86.41%. Tandem application of FC at early time-points and FGt detection by PCR at later time points seems to be a useful tool for long-term MRD monitoring in childhood ALL.

0021

INTRATHECAL LIPOSOMAL CYTARABINE IN CNS RELAPSE/ RESISTENCE OF PEDIATRIC ACUTE LEUKEMIA/LYMPHOMA: A MULTI-CENTER RETROSPECTIVE STUDY OF THE ITALIAN PEDIATRIC HEMATO-ONCOLOGY ASSOCIATION

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Background. The treatment of Central Nervous System (CNS) relapse/ resistence in pediatric acute Leukemia (AL)/lymphoma remains a challenging clinical problem. Liposomal Cytarabine (LC) (DepoCyte) is a new intrathecal (IT) formulation characterized by slow-releasing of free Cytarabine into the cerebrospinal fluid (CSF), resulting in longer drug exposure and possibly higher response rate. Severe neurotoxicity has been recently reported during LC treatment.

Table 1.

Patients' characteristics (N = 30).

Characteristic	No.
Gender:	
Male/Female	21/9
Median age at diagnosis (yrs) (range)	6.0 (0,3-17)
Median age at Liposomal Cytarabine treatment (yrs) (range)	9.4 (0,8-18)
Underlying neurological disease (Down's syndrome)	1
Diagnosis:	
Acute Lymphoblastic Leukemia (4 infant)	22
Acute Myeloid Leukemia (3 infant)	6
Non Hodgkin-Lymphoma	2
N. of CNS relapse at Liposomal Cytarabine treatment:	
First relapse	15
Second relapse	7
≥Third relapse	6
CNS Resistance	2
Type of relapse:	
Isolated CNS relapse/resistance	18
Combined relapse	12
Median Liposomal Cytarabine administration	4 (2-9)
N. of Liposomal Cytarabine administration for CR	3 (1-4)
Dose Level per patients and age:	
20 mg (< 1 years)	4
25 mg (1-3 years)	3
35 mg (3-14 years)	17
50 mg (> 14 years)	6
CNS response (%):	
Complete response	25 (83.4)
Partial response	3 (10.0)
Not Applicable	2 (6.6)
Neurological toxicity ≥ grade 3 (%):	4 (13.3)
Posterior reversible encephalopathy syndrome	1
Strabismus and clonie at right inferior limbs	1
Aphasia, Ataxia, hypostenia of left hemylate, corea	1
Partial seizures after CNS hemorrhagic stroke	1
Toxicity < grade 3 (%):	9(30.0)
Headache grade III	4
Irritability	3
Fever	2
Concurrent systemic HD-MTX/ARA-C (%)	21 (70.0)
Previous cranial RT/TBI (%)	8 (26.6)
Subsequent RT/TBI (%)	7 (23.3)
Subsequent SCT (%)	12 (40.0)
Outcome:	
Death from sepsis	2 (6.6)
Death from TRM	2 (6.6)
DOD	11 (36.7)
Alive in CCR	11 (36.7)
Alive in CR after subsequent relapse	4 (13.3)
Median follow-up (mths) (range)	30.5 (2-58)

CNS: central nervous system; HD-MTX/ARA-C: high dose Methotrexate/ Cytarabine; yrs: years; mts: months; CCR: complete continuous remission; CR: complete remission; RT: radiotherapy; TBI: total body irradiation; TRM: transplant related mortality; SCT: stem cell transplantation; DOD: died of disease

Aims. We retrospectively evaluated the safety and activity profile of IT LC in a cohort of 30 pediatric patients with CNS recurrence/resistance. **Methods.** From May 2005 to July 2011, 30 patients (21 males/9 females; median age 9.4 years) with CNS relapse/resistance (22 ALL, 6 AML and 2 NHL) were treated with IT LC at dosages ranging from 20 to 50 mg/dose depending on age, in eight AIEOP Centers. Patients concomitantly received oral administration of Dexamethasone (DEXA) at a dosage of 0.2 mg/kg twice a day for 5 days, associated with IT DEXA in ten children. Twenty seven out of 30 patients were simultaneously treated with systemic chemotherapy and 21 with concurrent high dose cytarabine or methotrexate. LC was started upon CNS relapse and was administered every 15 days, regardless of aplastic phase and underlying chemotherapy until CSF negativity in two subsequent lumbar punctures. Table 1 resumes the patients' clinical characteristics. LC treatment was discontinued when neurotoxicity appeared or in the presence of severe adverse events. Toxicity was evaluated according to Common Toxicity Criteria, version 3.0. **Results.** Twenty-five patients (83.4%) achieved a CNS cytological complete response (CR); three patients (10%), who presented radiological meningeal involvement at LC treatment, showed PR (negative CSF with persistence of less than 50% at neuroimaging) and two patients were not evaluated for IT pre-treatment. A median of four LC administrations were given (range, 2-9), with CSF negativity after three median IT administrations. Neurological toxicity > grade 3 was observed in 4 patients (13.3%); one patient experienced posterior reversible encephalopathy syndrome, the second had strabismus and clonus in the lower right limb, the third had partial seizures after CNS hemorrhagic stroke during the aplastic phase and the last one showed aphasia, ataxia, hypostenia of the left hemibody, corea. Two of these 4 patients resumed LC after CNS event resolution without complications. Other neurological adverse events < grade 3 were reported in 9 patients (30%), namely mild headache (n=4), irritability (n=3) and fever (n=2). No permanent sequelae were observed. Median overall survival was 20.9 months and the probability of being alive at 5 years was 45.7%. **Conclusions.** These encouraging data suggest that intrathecal IT LC is well tolerated and effective, as it can induce continuous CNS CR in a relevant proportion of children with relapsed/refractory leukemia/lymphoma. Despite its potential neurotoxicity, LC remains an interesting formulation since it reduces the frequency and total number of IT administrations. These characteristics associated with efficacy can improve compliance and quality of life in young patients. Further prospective studies on larger pediatric series are needed to confirm our observations and define optimal dosage and best timing administration of the drug in the pediatric setting.

0022

PROGNOSTIC SIGNIFICANCE OF IMMUNOHISTOCHEMICAL BIOMARKERS INCLUDING CD20, CD13 AND TDT IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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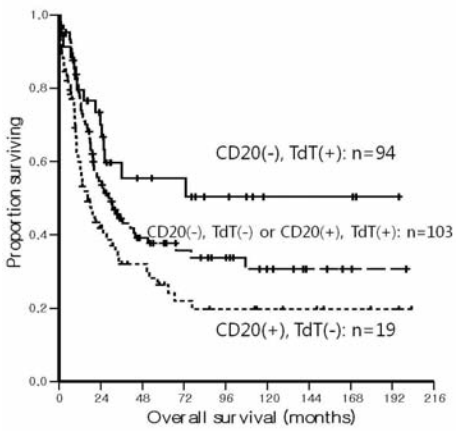
Background. Recently, targeted therapies with monoclonal antibody, such as rituximab which targets CD20 have been administered to the treatment of adult acute lymphoblastic leukemia (ALL), and promising results of reducing relapse rate and improving overall survival (OS) have been reported. Therefore, prognostic and predictive meanings of frequently-expressed cell surface markers of adult ALL have become important. **Aims.** We analyzed the prognostic significance of cell surface markers which are commonly expressed on the ALL cells for the treatment including allogeneic hematopoietic cell transplantation (allo-HCT). **Methods.** Patients who were diagnosed as ALL and treated in Asan Medical Center, Seoul, Korea, from 1995 until 2011 were included in this retrospective analysis. Patients with L3-type (Burkitt leukemia), lymphoid blast crisis of chronic myeloid leukemia were excluded. 'High risk' was defined as 'age≥35 years' or 'WBC count at diagnosis≥30x10^6/L' or 't(9;22) or BCR-ABL(+)'. Immunohistochemical markers were defined as positive when their expression was ≥20%. **Results.** Median age of 230 patients (male: female= 116: 114) included in this analysis was 38.0 (range 18-75) years, 28% of patients were Philadelphia-positive (Ph+), and 76% were high risk. Positivity of each immunohistochemical marker was 87.0% (CD19), 45.6% (CD20), 63.9% (CD22), 82.6% (CD10), 80.5% (terminal deoxynucleotidyl transferase; TdT), 66.8% (CD34), 39.2% (CD13), and 33.0% (CD33), respectively. Of 230 patients, 84% were treated with a combination chemotherapy consisting of vincristine, prednisolone, daunorubicin (VPD), and L-asparaginase, and 16% were treated with VPD plus tyrosine kinase inhibitor (imatinib, nilotinib or dasatinib). CR rate was 92%, which was not different between those with Ph+ and Philadelphia-negative (Ph-) ALL. AlloHCT was performed for 124 (54%) patients, during their first CR (80%) or more advanced stage. After 45.4 months of median follow-up period of surviving patients, 2-year and 5-year RFS was 48.3% and

36.2%, respectively. For overall survival (OS), 2-year and 5-year OS were 57.0% and 39.4%, respectively. When patients were divided into groups by prognostic factors, risk group (standard vs. high, 41.2 vs. 22.9 months, *p*=0.041), age (<45 vs. ≥45 years, 26.2 vs. 19.7 months, *p*=0.026), CD20 positivity (negative vs. positive, 30.8 vs. 19.3 months, *p*=0.007), CD13 positivity (negative vs. positive, 19.7 vs. 34.5 months, *p*=0.005), and TdT positivity (negative vs. positive, 17.5 vs. 30.7 months, *p*=0.05) significantly affected OS of patients. In the multivariate analysis with Cox-proportional hazard model, CD20 (HR 1.95, 95% CI 1.33-2.95, *p*=0.001), CD13 (HR 0.62, 95% CI 0.41-0.94, *p*=0.024) and TdT (HR 0.57, 95% CI 0.36-0.90, *p*=0.015) positivity were significantly associated with OS, as well as risk group (HR 1.76, 95% CI 1.11-2.79, *p*=0.017). When patients were categorized into 3 groups according to the positivity of CD20 and TdT, 5-year OS rate of each group was 36.1, 25.4, and 9.0 months, respectively (*p*=0.0003) (Figure 1). **Conclusions.** Positivity of common immunohistochemical cell surface antigen of adult ALL, especially of CD20, CD13, and TdT was an useful and discriminative prognostic biomarker.



Figuure 1. Overall survival curve according to the positivity of CD20 and TdT

0023

NORMAL KARYOTYPE PREDICTS BETTER SURVIVAL IN ADULT PH-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA - RESULTS OF THE RUSSAIN ACUTE LYMPHOBLASTIC LEUKEMIA(RALL)STUDY GROUP

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Background. Adult ALL is characterized by higher frequency of unfavorable biological features, slower molecular response, more toxicity followed by less compliance, translating in less efficacy compared with childhood ALL. So called "pediatric approach"applied in adult ALL is now considered to be more reasonable especially in the younger adults. **Aims and Methods.** RALL has initiated a prospective multicenter trial for adult Ph-neg ALL based on: 1) evaluation of b/m blast clearance after 7 days of Prednisolone (PRD) prephase and its substitution by Dexamethasone (DEXA) if b/m blast count was >25%; 2) continuous 2,5 years treatment schedule with prolonged L-asparaginase (Σ=590.000 IU); 3)evaluation of the impact of the late intensification (2 courses of HD of methotrexate and ARA-C) on MRD clearance. The study is registered on the ClinicalTrials.gov public site; NCT01193933. **Results.** From Nov, 2008, till Jan, 2012, 24 centers enrolled 150 pts: median age 28y (15-55), 62f/88m, 67%=B-lin, 33%=T-lin. Cytogenetics was evaluable in 60% of pts (n=90) and 47% of them (n=42) were with normal karyotype (NK). 26% of patients were in the standard risk (SR) group (WBC <30 for B-Lin, <100 for T-Lin, EGIL BII-III, T-III; LDH < 2N, No late CR, t(4;11)-negative), 59% - in the high risk (HR) group (WBC >30 for B-Lin, >100 for T-Lin, EGIL BI, T-I-II-IV; LDH > 2N, No late CR, t(4;11)-positive), 15% were not qualified. The analysis was performed in January, 2012.+8day b/m blast count was reported in 111 pts and has shown b/m blasts >25% in 64% of pts. The portion of non-responders to PRD was 50% in SR and 69% - in HR groups (p=0,04). In 118 analyzed pts CR rate was high in both risk groups (SR=94,1%; HR=91,0%) with total 7 induction deaths (5,9%) and 3 resistant leukemias(2,5%). L-asparaginase was stopped due to toxicity in 19% of patients, but it did not influence OS and DFS. With a median follow-up of 11 mo (1-36 mo) death in CR was reported in 6/108 (5,5%)pts, relapses - in 18/108 (16,6%). OS at 36 mo was 72,4%, DFS-60,5%.MRD analysis for clonal IgH and TCR rearrangements was carried out in 25 pts. And as in our previous studies (ASH 2006, abstr 2294) the clearance was slow with only 41,6% pts negative for MRD at day +133 (4mo) of the protocol. Two late intensification courses (day +157 = 5 mo) increased MRD negativity only up to 50%.Age, WBC, immunophenotype, LDH, risk group, +8 day b/m blast count, time without treatment (<>8days), L-asparaginase cessation did not influence survival. OS and DFS differed in pts with NK vs all other abnormalities: 84,6% vs 71,9%,(p=0,04) and 89% vs 70% respectively(p=0,03). **Conclusions**. So, our data demonstrated that in adult Ph-neg ALL normal karyotype is a favorable prognostic factor comparing to any abnormality and late intensification did not influence MRD clearance.

0024

WHITE MATTER ANISOTROPY AND NEUROCOGNITIVE OUTCOME IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA SURVIVORS TREATED WITH 3 DIFFERENT PROTOCOLS

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Background. Patients with childhood ALL achieve long-term disease-free survival yet neurocognitive outcome could affect the quality of life of survivors. The study aimed to assess the prevalence of neurocognitive dysfunction in survivors of childhood ALL treated with different protocols. **Patients and Methods.** Sixty ALL survivors aged 5-16 years; 2-9 years at diagnosis, CNS1, treated through 1998-2008 with chemotherapy only and regularly followed up in childhood cancer survivors clinic were compared to 60 healthy age and sex matched controls. Revision of scholastic achievement , type and risk of ALL, protocol of treatment, number, type and doses of intrathecal chemotherapy and high dose I.V methotrexate. Patients diagnosed between JAN 1998 to DEC 2000 were treated with Modified BFM 83; those between JAN 2001 to JUN 2004 with BFM 90 , and those from JUL 2004 to JUN 2008 with CCG 1991 for standard risk and CCG 1961 for high risk patients. Neurocognitive functions were tested using Wechsler Intelligence Scale for Children ,Benton visual retention (BVR) test and Trail making tests were done. MRI Brain was performed using diffusion weighed images and diffusion tensor magnetic resonance imaging (DTI). **Results.** Survivors treated with CCG protocol showed a significant decrease in all cognitive tests compared to control (p<0.05). BFM 90 group had a significant lower total IQ, verbal IQ, TMT-partA, compared to both control and Modified BFM 83 group, and a significant decrease in performance IQ, BVRT and TMT-partB compared to control. No significant difference in results of cognitive tests between Modified BFM 83 and control group. Both left and right frontal apparent diffusion coefficient (ADC) was significantly higher in CCG group compared to control BFM 90 and modified BFM 83 groups (p<0.05) and a significant decrease in fraction anisotropy (FA) of right frontal cortex in CCG group compared to control, BFM 90 and Modified BFM 83 groups (p<0.05). Right frontal FA was significantly lower in BFM 90 and Modified BFM 83 groups compared to control group. No significant correlation between IQ tests with age at diagnosis, length of treatment, duration from the end of therapy, number of IT doses. By comparing survivors according to duration since end of therapy, group I (1-2 years from end of chemotherapy) and II (2-5 years)of survivors showed a significantly lower full scale IQ, PIQ and correct BVRT compared to control group, and no significant difference in group III (5-10 years) compared to control ; left frontal ADC was significantly higher in group I and II of survivors compared to control and significant higher right frontal ADC in all three groups compared to control; left frontal FA was significantly lower in group I and III of survivors compared to control and the right frontal FA of survivors was significantly lower compared to control. **Conclusions.** CCG treated patients showed the worst neurocognitive outcome among three assessed protocols. Regressive course of neurocognitive dysfunction in chemotherapy-only treatment need to be prospectively addressed. Frontal lobe FA may be a useful marker for the assessment of neurotoxicity in childhood ALL survivors.

0025

ACUTE NEUROLOGIC COMPLICATIONS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. In recent years, 5 years event-free survival in children with acute lymphoblastic leukemia (ALL) has increased to 80% with the use of intensive chemotherapy protocols. It has been noted that the frequency of the side effects also increased. One of them is neurotoxicity reported in 3-13% of children with ALL. The most common neurological complications are convulsions, cerebrovascular accidents (bleeding, stroke), infections, posterior reversible encephalopathy (PRES), peripheral neuropathy and long term neurocognitive defects. **Aims.** This study analyzed acute central nervous system (CNS) complications occurred during ALL treatment. **Patients and Methods.** A total of 265 children diagnosed as ALL between 1995 and 2010 were enrolled into the study. They all completed the full courses of ALL-BFM-95 and ALL-BFM-2000 chemotherapy protocol. We retrospectively evaluated their clinical records and collected neurological events occurred during treatment. Patients with CNS leukemic infiltration and peripheral neuropathy at diagnosis and post-treatment late-onset encephalopathy or neurocognitive defects were excluded from the study. Neurological exam, cranial magnetic resonance imaging (MRI), electroencephalography (EEG), lumbar puncture (LP) and metabolic screening for differential diagnosis were performed. None of the patients had a history of neurological abnormalities prior to the onset of ALL. **Results.** Twenty-three(5%) out of 265 children (F: 13,M:10) had developed CNS complication during therapy. Their median age at diagnosis was 4.8±2.3 years (range, 1-17.5 years). None of them presented CNS leukemic involvement at diagnosis or at the time of the neurologic event. The events were convulsion(n:12), intracranial aspergillosis (n:4), PRES(n:3), sinus vein thrombosis (n:2), inadequate ADH syndrome (n:1) and transient ischemic attack (n:1). Ten children (43%) experienced neurologic events during the induction phase. In 8 cases (34%), the events occurred during reinduction and in 5 (21%), it occurred during the consolidation phase. Outcome of the 23 patients developed CNS complications, 14(60%) stayed healthy and 9 (39%) became epileptic. One (4%) died after the development of CNS complication. **Conclusions.** CNS complications are frequent events during ALL therapy, and require rapid detection and prompt treatment to limit permanent damage.

0026

SCREENING SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA FOR OBESITY, METABOLIC SYNDROME AND INSULIN RESISTANCE

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Bacground. Acute lymphoblastic leukemia (ALL) survivors have severe or life threatening chronic medical conditions 3.7 times more than their siblings. Among these medical conditions cardiovascular disease are quite frequent. **Aims.** Screening ALL survivors for obesity, metabolic syndrome (MS), insulin resistance (IR), hypothyroidism, insulin-like growth factor-1 (IGF-1), insulin like growth factor binding protein-3 (IGFBP-3) and prevention of cardiovascular disease by recommending healthy diets and increasing physical activity. **Methods.** Forty-four ALL survivors in first remission were enrolled. Twenty-six of them received 12-18 Gy cranial radiotherapy (RT). Patients' body mass indexes (BMI) at diagnosis and during the study were compared. BMI of the survivors were also compared with siblings. MS evaluation was performed in patients, parents and siblings older than six-years. HOMA index of the survivors was also calculated. In survivors with impaired fasting glucose levels, oral glucose tolerance test (OGTT) was performed. Thyroid functions, IGF-1 and/or IGFBP-3 levels of the irradiated survivors were also measured. **Results.** Median age was 6 years (2.5-17.5) at diagnosis and 11.5 years (6-23) during the study. Mean time following diagnosis was 5.4 (3-10) years. At diagnosis mean BMI percentile was 46.7 (3-95) and mean z score was -0.09±1.14; during the study these values raised to 71.1±25.6 (3-100) and 0.8±0.94, respectively (p<0.001). Twenty-five percent (n=11) and 48% (n=21) of the patients were obese/overweight at diagnosis and during the study respectively. At diagnosis only two patients (4.5%) had obesity whereas nine survivors (20%) had obesity (p=0.005). Survivors had significantly higher BMI-percentile and BMI-z score compared to their siblings (p=0.006 and p=0.011) (Figure 1). There was no correlation between survivors' obesity and obesity of the mother and father (p=0.3 and p=0.6 respectively). In three survivors (%6.8) there was MS. MS of the survivors had no correlation with maternal and paternal MS (P=0.1, P=0.5). In survivors exposed to RT, obesity/overweight ratio was 13/26 (50%) and MS incidence was 7.4%. Obesity/overweight and MS had no correlation with RT (p=0.7 and p=1). Among survivors exposed to RT, only three (11.5%) had thyroid dysfunction. Out of 26 survivors exposed to RT, 5 were obese and only one of these five survivors had thyroid dysfunction. There was no correlation between obesity and thyroid dysfunction (p=0.4). Out of 12 survivors with both IGF-1 and IGFBP-3 deficiency, 5(41.6%) were obese/overweight. There were no correlation between obesity/overweight and IGF-1 and IGFBP-3 deficiency (p=0.4). In 27.2% of the survivors there was IR and in 18% there was impaired fasting glucose. OGTT revealed abnormal glucose regulation and/ or IR in four of the survivors. **Summary and Conclusions. .** The incidence of obesity/overweight was significantly high in ALL survivors compared to their BMI at diagnosis and their siblings. Incidence of obesity was not correlated with RT, thyroid dysfunction and IGF-1 and IGFBP-3 levels. Incidence of MS was also higher than the normal population. Screening survivors for obesity, MS and IR and trying to improve these cardiovascular risk factors may prevent long-term morbidity and mortality.

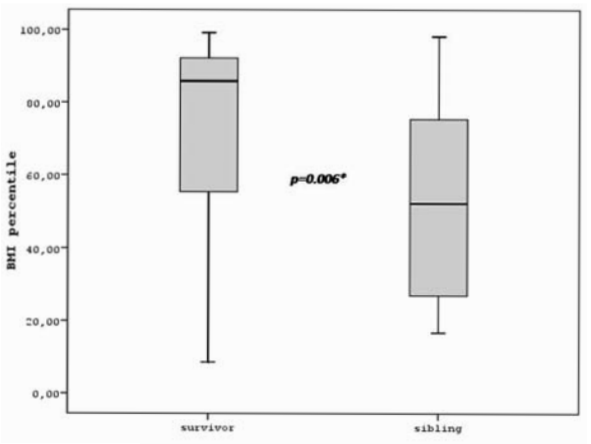

Figure 1. Body mass index of survivors and siblings.

0027

DELETIONS IN IKAROS BUT NOT ABERRANT CRLF2-EXPRESSION ARE ASSOCIATED WITH AN UNFAVORABLE PROGNOSIS IN BCRABL1 POSITIVE CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. In contrast to the high success rate to effectively treat children with precursor B-ALL, the prognosis of the 3% subset of *BCRABL1*-positive patients remains poor despite high-risk treatment regimens. Deletions in *Ikaros* (*IKZF1*) and high level of expression of *CRLF2* have been associated with a poor prognosis in childhood precursor B-ALL. However, whether these markers are also prognostic in *BCRABL1* positive ALL is unknown. **Aims.** We aimed to determine the prognostic value of *IKZF1*-deletions and aberrant *CRLF2*-expression in children with *BCRABL1* positive ALL since these markers may be of potential benefit to improve risk-stratification of these patients. **Methods.** Leukemic cells of newly diagnosed *BCRABL1* positive patients enrolled in Dutch Childhood Oncology Group studies were analyzed for *IKZF1* and *CRLF2*-status. The multiplex ligation dependent probe amplification (MLPA) p335-assay (MRC Holland) was used for discovery of *IKZF1*-deletions. Data were validated by array-CGH (Agilent, 180k chip) and a second MLPA-assay (p202, MRC Holland). *CRLF2*-expression level was determined by Affymetrix U133 plus 2.0 gene expression arrays. *BCRABL1* negative precursor B-ALL cases (n=558) served as reference. A high level of *CRLF2*-expression was assigned to 10% of all cases ranked by *CRLF2*-expression level. **Results.** Deletions in *IKZF1* were found in 62% of the cases (16/26) of which 12 with a partial deletion and 4 with a complete deletion of IKZF1. Patient characteristics revealed that *IKZF1*-deleted cases had a higher leukocyte count compared to unaffected cases (p=0.007). The cumulative incidence of relapse (pCIR) with death as a competing event indicated that *IKZF1*-deleted *BCRABL1*-positive cases had an increased risk of relapse compared to those with an unaffected *IKZF1*-gene (56.3% versus 12.5%, p=0.03).The *CRLF2*-expression was median of 703 arbitrary units (p25-p75:609-739) in *BCRABL1*-positive cases versus 683 arbitrary units (p25-p75:586-847) in the *BCRABL1*-negative cases (p>0.05). Hence, high expression of *CRLF2* cannot explain the poor prognosis of *BCRABL1*-positive cases. **Conclusions.** Our data suggest that a deletion of *IKZF1,* but not high expression level of *CRLF2,* can serve as unfavorable prognostic factor within

the subset of children with *BCRABL1*-positive precursor B-ALL. In a collaborative international study the independent prognostic value of *IKZF1*-deletions in *BCRABL1* positive ALL is currently being evaluated and will be presented at the meeting.

0028

OUTCOME OF HYPERFRACTIONATED CYCLOPHOSPHAMIDE, VINCRISTINE, DOXORUBICIN AND DEXAMETHASONE (HYPER-CVAD) CHEMOTHERAPY FOR 125 ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA IN AN ASIAN POPULATION

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Background. Hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (Hyper-CVAD) chemotherapy has been commonly used for adult patients with acute lymphoblastic leukaemia (ALL) in our institution. Earlier study from Kantarjian *et al.* reported complete response rate of 92% and 5-year survival rate of 38%. Clinical response data is lacking in Asian population. **Aims.** We report our data on the efficacy of hyper-CVAD chemotherapy regimen in adult patients with ALL and the baseline characteristics of these patients in our Asian population. **Methods.** Total 125 patients aged 18 years or above were diagnosed to have ALL from January 1998 to August 2010 and received hyper-CVAD chemotherapy regimen. Forty-seven patients underwent allogeneic haematopoietic stem cell transplantation (HSCT) and their survival data were censored at the time of HSCT. Clinical, haematologic and cytogenetic data were collected retrospectively. Treatment response and survival outcome were analyzed using SPSS version 17.0. **Results.** Median age of the cohort was 39 years (range 18-67). There were 100 Chinese, 18 Malays and 4 Indians. Complex cytogenetic aberrations, t(4;11)(q21;q23), low hypodiploidy and Philadelphia (Ph) chromosome were detected in 24%, 4%, 1% and 30% of patients respectively. T-cell phenotypes were detected in 12.8% of patients. Complete remissions (CRs) were achieved in 78.4% of patients. The median time to CR was 28 days. The presence of Ph chromosome predicted poor CR rate (62% versus 89% in Ph negative ALL; p=0.017). Twenty-two of 37 Ph positive ALL patients were given imatinib concurrently with hyper-CVAD and their CR rate was better (77% versus 40% in those who did not receive imatinib; p=0.012). Five patients (4%) died during the first 2 induction cycles due to infections. Relapses occurred in 32 patients. With median follow-up of 24 months (range 1-157), the estimated 5-year survival rate was 42%. Median overall survival was 21.3 months. The median survival was significantly influenced by cytogenetic risk categories (not reached for standard-risk, 10 months for Ph chromosome, and 11 months for other high-risk cytogenetics; p=0.016). Further analysis of Ph positive ALL did not reveal any survival benefit for patients who received imatinib (with median survival 10 months versus 8.7 months for those who did not receive imatinib; p=0.98) although imatinib improved the CR rate. Among 22 patients who received imatinib, 13 of them underwent HSCT following hyper-CVAD and imatinib. With an extended median follow-up of 51 months, an improved median survival (49.6 months) was observed in Ph positive ALL patients who underwent HSCT (p=0.002). **Conclusions.** Compared to earlier data, the CR rate of 78% was lower in our adult ALL patients who were treated with hyper-CVAD regimen. This may be accounted for by a higher percentage (30%) of Ph positive ALL in our series. The 5-year survival rate of 42% in our study was otherwise comparable to results from earlier studies. The survival outcome for high-risk cytogenetic subgroup including Ph positive ALL was disappointingly poor although imatinib and allogeneic HSCT were able to improve the overall long term survival in patients with Ph positive ALL.

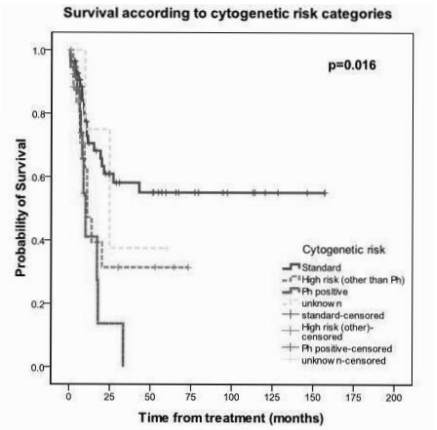

Figure 1. Survival according to cytogenetic risk categories.

0029

HONEY AND A MIXTURE OF HONEY, BEESWAX AND OLIVE OIL-PROPOLIS EXTRACT IN TREATMENT OF CHEMOTHERAPY-INDUCED ORAL MUCOSITIS: A RANDOMIZED CONTROLLED PILOT STUDY

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Objectives. In spite of being one of the most investigated subjects among supportive care in cancer, no therapy has been found effective in treatment of chemotherapy-induced oral mucositis. Based on the observations that honey bees products have anti-inflammatory and wound healing effects, the present study tried to evaluate the effect of topical application of honey and a mixture of honey, olive oil-propolis extract and beeswax (HOPE) in treatment of oral mucositis. **Methods.** This was a randomized controlled clinical trial conducted on 90 patients with acute lymphoblastic leukemia and oral mucositis grades 2 and 3. The mean age of enrolled patients was 6.9 yr. The patients were assigned into three equal treatment groups: Honey, HOPE and control groups. Topical treatment for each patient consists of honey, HOPE and benzocaine gel for honey HOPE and control groups, respectively. **Results.** Recovery time in grade 2 mucositis was significantly reduced in the honey group as compared with either HOPE or controls ($p < 0.05$). In grade 3 mucositis, recovery time did not differ significantly between honey and HOPE ($p=0.61$) but compared with controls, healing was faster with either honey or HOPE ($p<0.01$). Generally, in both grades of mucositis, honey produced faster healing than either HOPE or controls ($p<0.05$). **Conclusions.** Based on our results which showed that honey produced faster healing in patients with grade 2/3 chemotherapy-induced mucositis, we recommend using honey and possibly other bee products and olive oil in future therapeutic trials targeting chemotherapy-induced mucositis.

Table 1. Recovery time among studied patients.

	Recovery time (days) Mean ± SD			p		
Grade of mucositis	Group 1 (honey)	Group 2 (HOPE)	Group 3 (control)	Honey vs HOPE	Honey vs control	HOPE vs control
2	3.6 ± 0.8	4.2 ± 0.7	4.6 ± 0.9	0.0324	0.0017	0.1750
3	5.4 ±1.1	5.8 ± 2.6	8.6 ± 1.0	0.6108	0.0001	0.0012
2 and 3	4.25 ±1.25	5.75 ± 2.57	6.10 ± 2.47	0.0056	0.0005	0.5928

P < 0.05 is significant

P < 0.01 is highly significant

0030

EFFICACY OF INTRATHECAL LIPOSOMAL CYTARABINE IN TREATMENT OF CHILDHOOD HEMATOPOIETIC MALIGNANCIES WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT - EXPERIENCE OF POLISH PEDIATRIC LEUKEMIA/LYMPHOMA STUDY GROUP

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Background. Liposomal cytarabine for intrathecal administration is characterized by prolonged activity and better penetration to central nervous system

(CNS). This makes it promising medicine for treating children with hematopoietic malignancies relapsing in CNS or with refractory CNS disease. **Aims of the study**. The study aimed at retrospective evaluation of the effectiveness of liposomal cytarabine (Depocyte®) administrated intrathecally as a part of the treatment of hematopoietic malignancies in Polish children and adolescents. **Patients and Methods.** The study group consisted of 24 patients, 13 boys and 11 girls, treated in the centers of Polish Pediatric Leukemia/Lymphoma Study Group, including 19 patients with acute lymphoblastic leukemia (ALL), 3 patients with acute myeloid leukemia (AML) and two children with high grade Non-Hodgkin's Lymphomas (NHL). The median age of the children was 10.9 years (range: 1.3 to 18 years). Liposomal cytarabine treatment was administered on compassionate basis to 21 children with relapsed acute leukemia / NHL, a single child with secondary leukemia, one patient with severe neurotoxicity after intrathecal Methotrexate during front-line treatment and in one child with large granulocytic sarcoma, penetrating into CNS. Fourteen patients received liposomal cytarabine dosage of 50 mg, while the remaining 10 children were exposed to the doses of 25-35 mg, all in association with prophylactic dexamethasone administration. The number of liposomal cytarabine injections ranged from 1 to 11, mean 5 doses per patient. **Results.** The clearance of CNS disease was achieved in 17 of 24 patients (71%). Eleven children were alive during the follow-up procedure, including 7 patients in complete remission after treatment completion. Grade IV neurotoxicity was observed in five children, which might be also partly related to CNS malignancy. Another side effects occurred in 4 patients, including headache, vertigo, paresthesias and seizures. **Conclusions.** Liposomal cytarabine administered intrathecally is effective treatment for CNS disease in children with relapsed acute leukemia/NHL with acceptable toxicity profile.

0031

ASSESSMENT OF HEALTH RELATED QUALITY OF LIFE IN CHLDREN WITH CANCER DURING THERAPY

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Background. the increased survival o cancer patients may be accompanied by long term burden for some individuals related to the unique characteristics of their cancer diagnoses and its treatment on their educational, psychological and social development. Thus the identification of subgroups of childhood cancer survivors at risk for poor HRQoL is important for development of new strategies as well as determination of treatments promoting long term survival and reducing risk for poor HRQoL outcomes will be a goal. **Aims.** to assess quality of life (QoL)of children with newly diagnosed cancer at one and three months of starting therapy, and to obtain effects of sex, age, type of malignancy, stage of the disease and treatment on quality of life of those children. **Patients and Methods.** A descriptive study was carried out on the newly diagnosed children with cancer during treatment at Pediatric Hematology Oncology Unit, Zagazig University Children Hospital during period between Jan 2011 and Jan 2012. In this study we use PCQL-32 questionnaires to measure HRQoL in pediatric cancer patients. This scale defines HRQoL in five items that measures impact of disease and treatment on the individual's physical, social, psychological, cognitive functioning and disease/treatment -related symptoms. It assesses both patient's and parent's perceptions of child' HRQoL. **Results.** This study was conducted on fifty newly diagnosed cancer patients, their mean age was 9±2.5 years old, 54% of them were males and 46% females. Thirty patients were diagnosed as leukemia, while twenty cases as solid tumors. Eighty % of our patients were received chemotherapy, while 14% were received Radiotherapy and only 6% received combined therapy. QoL of children at three months was significantly better than QOL at one month of therapy in all functioning and there was Strong correlation between child and mother report at one and three month in all functioning of QOL. No significant difference between two types of malignancy and child QoL at one and three months of therapy. There was a better quality of life in children received radiotherapy only than other types of treatment at one month, while at three month children received only chemotherapy had better QoL. There was significant difference between age groups in cognitive function, with better functioning in the youngest age at one and three months of therapy. There was no significant difference between both sex at 1&3 months. There was a significant difference among birth order groups with worst of the 4th or more order. QoL of children to the youngest mothers and highly educated parents was better than those to older mothers or low educated parents respectively. **Conclusions.** HRQoL in childhood cancer survivors mainly affected by time elapsed since diagnosis, stages of cancer and type of treatment while most of sociodemographic factors has no or little effect which limited to one month only, this indicates that to improve QoL of those children, we have to improve their treatment and psychological support for both children and parent to facilitate their coping with disease and its treatment.

Acute myeloid leukemia - Biology 1

0032

ADVERSE PROGNOSTIC IMPACT OF ABNORMAL LESIONS DETECTED BY GENOME-WIDE SINGLE NUCLEOTIDE POLYMORPHISM ARRAY KARYOTYPING ANALYSIS IN *de novo* ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE

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Background. Acute myeloid leukemia with normal karyotype (AML-NK) corresponds to a heterogeneous disease with diverse clinical outcomes, thus requiring an efficient risk-stratification. Recently Single-nucleotide polymorphism array (SNP-A) analyses have revealed previously unrecognized copy number abnormalities (CNA) and uniparental disomy (UPD) that might be helpfull for a better risk-stratification in this subset of AML. **Aims.** Analysis of the prognostic significance of SNP-A-based karyotyping in a homogenous treated cohort of older AML-NK (50-70 years) with a previously untreated *de novo* AML. **Patients and Methods.** We performed Affymetrix SNP6.0 array analysis in a cohort of 117 AML-NK enrolled in the randomized multicentric phase 3 ALFA0701 trial (NCT00927498) comparing induction with daunorubicine and cytarabine without (DA-arm) or with Gemtuzumab-Ozogamicin (GO)(DAGO-arm). **Results. :**Median age of our patients was 61 years (range, 50 to 70 years) and 54 patients (46%) were male and 63 (54%) were female. We demonstrate the presence of 69 cryptic genomic aberrations in 47 patients (40%) consisting of 13 deletions (13 patients), 11 gains (11 patients) and 45 regions of UPD (33 patients). The median size of deletions was 2 Mb (0.15Mb-18Mb) and of gains was 0.5Mb (0.12-1.4Mb).The median size of UPD's was 42 Mb (3Mb-158Mb). Chromosome 13(q12.11-q34) and 1(p36.31-p36.21) were the most frequently affected with UPD observed in 8 and 5 patients, respectively. All 8 patients with UPD of chromosome 13 had this occurrence in sub-clones and all harbored *Flt3-ITD*. There were no significant correlations between the presence of genomic aberrations and age, sex, WBC, or mutations *in NPM1, CEBPA, TET2, IDH1*/2, *FLT3*-ITD or *DNMT3a*.The median duration of follow-up from time of diagnosis of the study group was 17.5 months (range 1-43). Patients with abnormal SNP-A lesions compared to those without, did not show a significant different complete remission rate (87% vs 93%;p=0.34) or a 2 year cumulative incidence of relapse (56.6% vs 56.9%;p=0.46). Resulting EFS did not differ significantly in patients with abnormal SNP (HR=1.52, 95%CI:0.93-2.48; p=0.10). By contrast, overall survival (OS) was shortened in AML with SNP-A lesion (HR=1.85, 95%CI:1.02-3.34;p=0.043). Patients with number of abnormalities (≥2) had a highly worse OS (HR=2.41, 95%CI:1.15-5.06; p=0.020) and EFS (HR= 2.41,95%CI:1.25-4.63; p=0.008). DA-arm when compared to DAGO-arm, have a significantly shorter OS (HR= 0.54, 95%CI: 0.29-0.99; p= 0.046) and EFS (HR=0.50, 95%CI 0.30-0.83; p=0.007). In DA-arm we found a very poor prognosis impact of SNP-lesions on both OS (HR= 2.93, 95%CI:1.33-6.46; p= 0.008) and EFS (HR= 2.89, 95%CI:1.45-5.78;p=0.003). By contrast, DAGO-arm erase the prognostic value of the SNP lesions on both OS (HR= 1.49, 95%CI: 0.60-3.72; p= 0.39) and EFS (HR=1.26, 95%CI: 0.59-2.68; p= 0.56). Similarly none genes mutations studied (*NPM1, CEBPA, TET2, IDH1*/2, *FLT3*-ITD, *DNMT3a*) impact on OS and EFS of DAGO-arm patients. Very interestingly a significantly shorter EFS in DAGO-arm was restricted to patients with ≥2 SNP-A abnormalities (HR=2.58, 95%CI: 1.08-6.13; p=0.032). **Conclusions.** We conclude that SNP-A Karyotyping improve outcome prediction in older AML-NK. In particular a number of SNP-A abnormalities ≥2 is a strong poor prognosis marker.

0033

LYSOPHOSPHATIDIC ACID (LPA) ENHANCES MOTILITY AND PROLIFERATION OF HEMATOPOIETIC STEM CELLS AND CONTRIBUTES TO THE PATHOGENESIS OF ACUTE MYELOID LEUKEMIA (AML)

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Activating mutations of the FLT3 receptor tyrosine kinase, especially the internal tandem duplication (FLT3-ITD) are among the most common abnormalities in adult AML. These mutations induce ligand independent constitutive activation of the receptor and downstream signaling pathways, including RAS/ERK, PI3K and STAT5-signaling. Several lines of evidence suggest that FLT3-ITD

mutations induce an aggressive disease, characterized by a high rate of disease recurrence and poor clinical outcome.To better understand the specific phenotype induced by FLT3-ITD mutations, we performed microarray expression analysis of primary leukemic samples from patients with different types of FLT3-mutations. One gene specifically upregulated in FLT3-ITD+ AML was *ENPP2* coding for Autotaxin (ATX). The ATX protein acts as a secreted lysophospholipase D (lysoPLD) by converting lysophosphatidylcholine (LPC) to the lipid mediator lysophosphatidic acid (LPA), which signals via G-protein coupled LPA receptors (LPAR1-6) and has important functions in cell migration and proliferation. The current study demonstrates that ATX expression is specifically upregulated and functionally active in acute myeloid leukemia (AML) harboring an internal tandem duplication (ITD) mutation of the FLT3 receptor gene.ATX expression was evaluated in different leukemic cell lines and hematopoietic stem cells using RT-Q-PCR, Western blot, and functional assays. Chemotaxis was studied with transwell migration assays and clonogenic potential was determined with colony-forming assays. Results were verified by retroviral transduction of ATX into ATX-negative THP-1 cells. In addition, the effect of ATX inhibition was evaluated using a novel ATX-specific inhibitor, ACD-0708.ATX was found to be highly expressed in AML cells, especially in cells harboring FLT3-ITD. Comparable ATX expression levels were also found in CD34+ cells, whereas mature cells showed significantly lower ATX expression. ATX-expression led to increased migration towards LPC, whereas ATX neg. cells showed no significant response. LPC-mediated migration in MV4-11 cells could be blocked by PKC412, a tyrosine kinase inhibitor, indicating the causative role of FLT3-ITD. Moreover, LPC-induced chemotaxis was blocked upon JUN dephosphorylation and inhibition of ATX using the small molecule inhibitor ACD-0708, respectively. LPA increased chemotaxis in human leukemic cell lines and CD34+ progenitors in a dose dependent manner by at least 50%. The LPC/LPA-induced chemotaxis in MV4-11 cells was sensitive to inhibition by pertussis toxin and Ki16425, a LPAR1/3 selective LPA-inhibitor. The observation that LPA3 was not expressed in MV4-11 cells indicates that migration is dependent on the presence of LPAR1. Moreover, ATX expression increased colony-forming capacity in the presence of LPC or LPA by 40% and 75%, respectively. Vice versa, inhibition of ATX using ACD-0708 selectively induced killing of ATX-expressing cell lines.Our data indicate that the ATX-LPA axis contributes to proliferation and migration of hematopoietic stem cells and that aberrant ATX expression may support the pathogenesis of FLT3-ITD positive AML.

0034

DEEP SEQUENCING REVEALS ALLELE-SPECIFIC HYPERMETHYLATION AT 14Q32 IMPRINTED DOMAIN IN ACUTE PROMYELOCITIC LEUKAEMIA (APML)

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BackgroundEmerging evidences have showed that epigenetic alterations, including DNA methylation, occur at a very early stage of the tumorigenic process. Therefore the identification of specific loci that undergo DNA methylation changes might identify genes and factors important for disease progression. In chromosome 14q32, the DLK1/DIO3 domain harbors differentially methylated regions (DMRs) that regulate the expression of imprinted genes by the modulation of the allelic CpG methylation pattern. The intergenic DMR (IG-DMR), located between the genes DLK1 and MEG3, is the imprinting control region (ICR) and operates hierarchically on the secondary MEG3-DMR that resides in the MEG3 promoter. Previous studies showed that a subset of microRNAs clustered downstream MEG3 and regulated coordinately with MEG3 is overexpressed in APML only.AimsThe aim of the study is to determine whether the methylation profile of IG-DMR and MEG3-DMR is altered in APML leading to overexpression of miRNAs. Furthermore, since DNA methylation is differentially distributed between the two alleles, we aim to obtain the allelic methylation pattern of the DMRs.MethodsNine regions overlapping IG-DMR and MEG3-DMR were selected for high-throughput amplicon sequencing with Roche 454 GS FLX Titanium. Amplicon libraries were obtained from bisulfite treated genomic DNA from APML cases, including diagnostic and remission sample pairs, healthy donors and additional acute myeloid leukaemia (AML) subclasses. Sequence output was analysed with QUMA (http://quma.cdb.riken.jp). UCSC database (http://genome.ucsc.edu/) was used to search for SNPs among the regions selected.ResultsWe generated 1,093,266 sequence reads with an average read length of 399 bp. Reads were aligned to the germline sequence and, after filtering out the reads that did not meet the quality criteria, 923,981 sequences were used to determine the methylation status of a total of 202 CpGs. Unsupervised hierarchical clustering showed that APML was predominantly hypermethylated as compared to normals. Pair wise T-test performed between diagnosis/remission APML sample pairs confirmed that the onset of the disease was associated with increase of DNA methylation in 14q32. CpGs affected by aberrant methylation were not randomly distributed across the regions, but organized in a specific pattern included in the MEG3-DMR. Notably, statistical analysis performed on the IG-DMR did not provide any evidence of DNA methylation changes affecting the ICR. We next identified heterozygous SNPs in the selected regions and conducted allele-specific methylation analysis. Results showed that hypermethylation arises in a mono-allelic manner, retaining a conservative pattern on the other allele (Figure 1). **Summary and Conclusions. .** APML is associated with allele-specific hypermethylation at the MEG3-DMR, a regulatory region embedded in DLK1/DIO3 imprinted domain. In contrast, the methylation profile of the ICR remains unchanged. These findings indicate that in APML aberrant epigenetic profile might lead to altered expression of regulated genes without imprinting disruption.

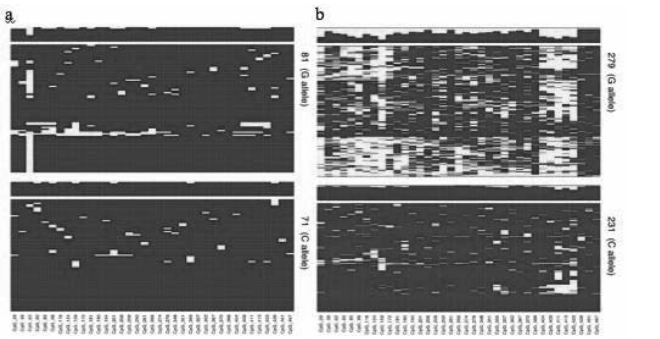

Figure 1. Allele-specific hypermethylation in APML. a) diagnosis; b) complete remission. Grey, methylated; white, not methylated.

0035

THE EFFECTS OF HISTONE DEACETYLASE INHIBITORS ON THE MALIGNANCY OF T(6;9)-DEK/CAN-POSITIVE LEUKEMIA

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Background. Acute myeloid leukemia (AML) is characterized by an abnormal accumulation of hematopoietic progenitors in the bone marrow (BM). The AML phenotype is maintained by an accelerated proliferation due to a differentiation block that prevents progenitors from reaching the post-proliferative stage of blast cells. This is supported by the aberrant stem cell capacity of poorlydefined leukemic stem cells (LSC). Specific chromosomal translocations, such as t(8;21), t(15;17), t(6;9) represent the leukemia initiating event. The related AML associated fusion proteins (AAFPs) such as PML/RAR, AML-1/ETO or DEK/CAN recapitulate the leukemic phenotype *in vitro* and *in vivo*. Most of the AAFP interfere with the epigenetic regulation of transcription by modifying key processes of chromatin modeling such as histone acetylation and methylation as well as DNA methylation. In the DEK/CAN fusion protein all the chromatin binding domains of DEK are conserved and we recently showed that DEK/CAN is associated to chromatin and strongly interferes with chromatin modeling by inhibiting the decondensation of chromatin and accessibilityto transcription. **Aims.** Here we investigated, whether it is possible to revert the leukemogenic potential of DEK/CAN by histone-deacetylase (HDAC) inhibitors (HDACi) such as Valproic acid (VPA), Dacinostat and Vorinostat. **Methods.** The effects of the HDACi on histone acetylation and methylation were studied by Western blotting and intracellular FACS on DEK/CAN-expressing human U937 cells. Modifications of the differentiation potential and the stem cell capacity was assessed in retrovirally transduced primary murine HSC in liquid culture, by replating efficiency and colony forming unit spleen day 12 (CFU-S12) assay, respectively. Furthermore we used a mouse model of DEK/CAN-positive leukemia and a xenograft model based on human t(6;9) positive cells inoculated in NSG mice. **Results.** Here we show that i.) DEK/CAN interferes with thee chromatin decondensation by Dacinostat in U937 cells; ii.) HDACi with the exception of VPA induce differentiation of DEK/CAN-positive murine HSC and reduced their replating efficiency; iii.) Dacinostat most efficiently as compared to the other HDACi abolished the stem cell capacity of DEK/CAN-positive leukemic stem cell as revealed by a strongly reduced number of colonies in a colony forming unit spleen day 12 assay (CFU-S12), in which DEK/CAN was not anymore detectable even by RT-PCR upon exposure to HDACi; iii.) exposure to the HDACi prevented leukemia induction in the xenograft model, without interfering with the engraftment. **Summary.** Our here presented results suggest that the HDACi even with different efficacy are able to reveert with the malignancy of DEK/CAN-positive leukemic cells.

0036

GENOME-WIDE GENOTYPING OF *de novo* AND THERAPY-RELATED ACUTE MYELOID LEUKEMIA WITH T(9;11)(P22;Q23) REVEALS NOVEL RECURRENT GENOMIC ALTERATIONS

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Background. Rearrangements of the mixed-lineage leukemia (*MLL*) gene play an important role in the development of acute leukemia. In acute myeloid leukemia (AML), translocation t(9;11)(p22;q23) *MLLT3-MLL* is the most common genetic alteration involving *MLL.* Translocation t(9;11) can be found in *de novo* AML as well as in therapy-related AML (t-AML). Clinically, t(9;11)-associated AML is characterized by a broad heterogeneity and secondary genetic events have been discussed to underlie these heterogeneous phenotypes. **Aims and Methods.** To identify additional genetic alterations in t(9;11)-positive AML, we performed single nucleotide polymorphism (SNP)-array analysis of 40 diagnostic leukemia samples [*de novo* AML: n=22; t-AML: n=14, unknown: n=4]. Control-DNA from remission bone marrow or peripheral blood was available for paired analysis in 15 cases (38%). Data were processed using reference alignment, dChipSNP, and circular binary segmentation. In addition, gene mutation/expression status was determined for a set of genes, known to be frequently altered in AML (*FLT3* [ITD and TKD], *NPM1*, *IDH1*/2, *DNMT3A*, *TET2*, *NRAS*, and deregulated expression of *EVI1*). **Results.** Paired analysis revealed a mean of 1.9 somatic copy number alterations (CNAs) per case (range: 0-12); 50 % of cases lacked any CNAs. There were no significant differences in the mean number of CNAs between *de novo* and therapy-related cases [*de novo* AML: 1.1 (range: 0-4); t-AML: 2.8 (range: 0-13); *p*=0.78]. Recurrent deletions were detected at chromosomal bands 7q36.1-36.2 (n=2; *de novo*: n=1; t-AML: n=1) and at the chromosomal translocation breakpoint 11q23 (n=4; *de novo*: n=3; t-AML: n=1). The del(7)(q36.1-36.2) partly overlapped with the minimally deleted region that we previously identified in 8% of core-binding factor AML. The only gene located in both regions was *MLL3*, a member of the mixed-lineage leukemia gene family. The most recurrent gain was trisomy 8 [n=8 (20%)], which was also detected by conventional cytogenetics in all cases. Trisomy 8 was more commonly found in *de novo* AML than t-AML (6/22; 27% vs. 1/14; 7%). Novel recurrent focal gains were identified at 9p22.1 (n=2; size: 341 Kb) and at 13q21.33-q22.1 (n=2; size: 1021 Kb) with each region harboring genes potentially involved in cancer pathogenesis (*ACER2* in 9p; *KLF5* in 13q). Notably, no copy number neutral loss-of-heterozygosity (CN-LOH) was detected in the 40 cases. Mutation analysis revealed mutations in *FLT3* (ITD; 2/36 (6%); TKD; 3/29 (10%); *NPM1* 2/31 (7%), and deregulated *EVI1* expression in 8/16 (50%) patients; none of the cases showed mutations in *IDH1*/2 [0/29], *DNMT3A* [0/19], *TET2* [0/15], and *NRAS* [0/6]. **Summary and Conclusions.** AML with t(9;11) is characterized by remarkably few CNAs; we found no substantial differences between *de novo* and therapy-related cases with regard to lesion-/mutation type or frequency. Nevertheless, we were able to identify new recurrent lesions as e.g. deletions in 7q36.1-36.2. Determining their functional role in leukemogenesis and drug resistance will provide new insights into the pathogenesis of t(9;11)-rearranged AML.

0037

PHENOTYPICAL AND FUNCTIONAL ABNORMALITIES OF MESENCHYMAL STEM CELLS FROM ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Contribution of stromal cell compartment to the leukemogenesis process remains controversial. Thus, although abnormalities of stromal cells (which do not belong to malignant clone) have been reported in acute myeloid leukemias (AML), recent data from animal models suggest that genetic changes in stromal cells can induce myelodysplastic syndromes and secondary leukemias (Raaijmakers et al. Nature 2010;464: 852-7). **Aims.** In the present work, we studied the phenotypical and functional properties of mesenchymal stem cells (MSCs) from AML patients compared to normal controls. **Methods.** Bone marrow-derived MSCs have been obtained from 12 AML patients (5 AML0/1, 3 AML3, 4 AML4/5) and from 6 normal controls (harvested during orthopedics surgery). MSCs were cultured in alphaMEM medium supplemented with 10% FCS and 1ng/mL FGF2. The following analyses have been performed : proliferative capacity (evaluated by population doubling time or PDT), cloning efficiency (evaluated by CFU-F assay), immunophenotypical characteristics (CD45, CD34, CD14, CD90, CD73, CD105, CD106, CD271, CD146, CD31, CD309, CD49d, CD49e), mesenchymal differentiation capacity (towards adipocytic, osteoblastic and chondrocytic lineages), and secretion capacity of essential niche factors including SDF-1 (CXCL12), SCF (stem cell factor) and Ang-1 (angiopoietin-1). All these analyses have been carried out at the end of the 2nd passage. **Results.** While no evident differentiation deficiency towards adipocytic, osteoblastic or chondrocytic pathways was found, a clear decrease of CFU-F frequency was noted in most of the AML patients (8/12 patients). These 8 patients (AML-Clono^{low}) were present in all FAB groups and did not display any particular feature, considering age, sex, cytogenetic and molecular, abnormalities, or clinical evolution. Compared to normal MSCs, MSCs/AML-Clono^{low} tended to display prolonged PDT (p=0.071) and were associated with lower expression levels of VCAM-1 (p=0.014). Compared to MSCs/AML-Clono$^{norm/high}$, MSCs/AML-Clono^{low} displayed decreased PDT (p=0.041) with decreased secretion capacity of SDF-1 (p=0.007) and in a lesser extend of Ang-1 (p=0.027) without clear change in SCF secretion (p=0.126). **Summary and Conclusions.** Our study showed that BM-derived MSCs from most of AML patients display proliferative and clonogenic deficiency. These abnormalities are associated with a decreased expression of niche factors which are essential for hematopoietic stem cell control. To better characterize MSCs from AML patients, large and middle scale studies of gene expression profile are in progress together with their normal and leukemic hematopoiesis supportive capacity.

0038

MIR-155 REGULATIVE NETWORK IN FLT3 MUTATED ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML)represents a heterogeneous disorder with recurrent chromosomal alterations and molecular abnormalities. Among AML with normal kariotype (NK-AML)FLT3 activating mutation, internal tandem duplication (FLT3-ITD), is present in about 30% of patients, conferring unfavorable outcome. Our previous data demonstrated specific up-regulation of miR-155 inFLT3-ITD+ AML. miR-155 is known to be directly implicated in myeloid hyperplasia and/or hematopoiesis. Our aim was to integrate data from different source as GEO gene expression database and MIR@NT@N prediction tool to achieve a model about the role of miR-155 inFLT3-ITD+ AML and to validate this by experimental analysis. In this study we applied a four steps strategy. At the first step, using gene expression dataset from GEO database, we generated the transcription factors co-regulation network acting in FLT3 mutated AML and at the same time, we predicted the miR-155-TF connections by MIR@NT@N. In the second step, we extracted, from the general network, the module of transcription factors connected to miR-155. At the third step, we compared the mir-155 module with the canonical pathways. At the final step, using a new cohort of newly diagnosed AML patients, we verified the expression levels of most intriguing hubs and correlated them to miR-155 expression levels. From these analyses, we derived a sub-network, called “miR-155 module”that describes functional relationship among miR-155 and transcription factor in FLT3-ITD+ AML. We confirmed a strong up-regulation of miR-155 in the FLT3-ITD+ AML. We found that “miR-155 module”is characterized by the presence of six transcription factors as central hubs: four miR-155 regulators (JUN, RUNX1, FOSb, JUNB) and two targets of miR-155 (PU.1, CEBPB) all known to be “master”genes of myelopoiesis. We found, in FLT3-ITD+ AML, a significant down-regulation of miR-155 target genes *CEPB-beta* and *PU.1* (respectively 0.354 fold p=0.000 and 0.404 fold p= 0.000) and up-regulation of miR-155 regulator genes JUN and RUNX1 (2,597 fold p=0.0210 and 2,64 fold p<0.0001 respectively). We described, for the first time, a regulatory pathway that connects FLT3-ITD mutation, a poor prognostic marker for AML, to reduced expression of TFs master regulators of myelopoiesis. Our results suggest that activating mutation of FLT3 in AML can lead, through the induction of JUN, to increased expression of miR-155, which then causes down-regulation of *PU.1* and *CEBP-beta* and consequently causes block of myeloid differentiation. More simply, FLT3- ITD → ↑JUN → ↑miR-155 → ↓PU.1 ↓CEBPbeta → ↓Myelopoiesis. In conclusion our study consolidates data on miR-155 association with FLT3-ITD+ AML, describes an integration of sequence-based prediction analysis with expression network that individuates vertices involved in the molecular pathogenesis of FLT3 mutated AML, suggests a molecular pathway

that starting from in FLT3 activating mutation, through miR-155, damages myeloid differentiation. We also suggest that miR-155 deregulation may act as central hub in the multi-steps mechanism of FLT3 mutated leukemogenesis offering new therapeutic strategies.This work was supported by a grant of Associazione Italiana Ricerca sul Cancro (Project IG 10701 AIRC)

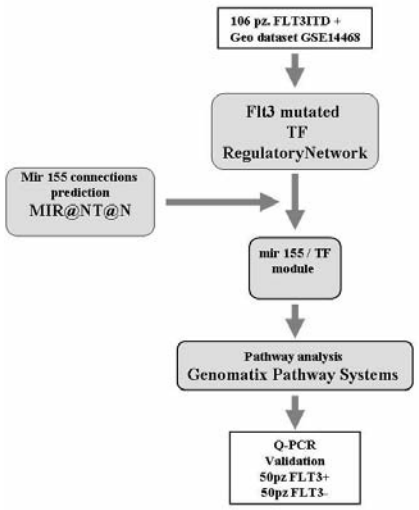

Figure 1. The study schema and workflow is shown

0039

DIFFERENTIAL DNA METHYLATION IN AML PATIENTS WITH WILD-TYPE AND MUTATED DNMT3A AND PROGNOSTIC IMPACT OF DNA METHYLATION

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Introduction. DNA methylation has been established as a mechanism playing an important role during the process of leukemogenesis. Mutations in *DNA methyltransferase 3A (DNMT3A)* gene have been found in patients with acute myeloid leukemia (AML) and have been shown to be associated with adverse clinical outcome. **Aims.** We studied the relationship between DNA methylation status of selected genes and *DNMT3A* mutational status and also the impact of DNA methylation on the patients' prognosis. **Patients and Methods.** We examined 79 AML patients at diagnosis (intermediate and high cytogenetic risk) and 20 healthy donors for the presence of aberrant DNA methylation of 12 tumor suppressor genes (TSG) (*CDKN2B*, *CALCA*, *CDH1*, *ESR1*, *SOCS1*, *MYOD1*, *DAPK1*, *TIMP3*, *ICAM1*, *TERT*, *CTNNA1*, *EGR1*) by MethyLight and for mutations in the gene *DNMT3A* by direct sequencing. We also studied methylation status of 24 HOX genes using methylation-restriction endonucleases followed by RQ-PCR arrays in 10 AML samples compared to 4 healthy donor samples. **Results.** Sequencing of cDNA between amino acids 300 and 930 revealed that 32 of 79 AML patients had *DNMT3A* mutation. MethyLight assessment of 12 TSG showed the following frequencies of hypermethylation: *CDKN2B* (47%), *CALCA* (43%), *CDH1* (22%), *SOCS1* (24%), *MYOD1* (18%), *ESR1* (14%). The remaining 6 genes were weakly methylated in less than 10 % AML patients at diagnosis and were therefore excluded from further analysis. Comparing overall cumulative DNA methylation levels and numbers of simultaneously hypermethylated (HM) genes to mutational status of *DNMT3A* gene, we found lower levels of DNA methylation (P<0.0001) as well as lower numbers of concurrently hypermethylated genes (P<0.0001) in patients with *DNMT3a* mutations. We observed the same trend also in DNA methylation levels of HOX genes when comparing AML patients with mutated (n=4) versus wild-type (n=6) *DNMT3A*. Furthermore, DNA methylation levels or numbers of HM genes were independent of age, gender, percentage of blasts (PB and BM) or cytogenetic risk group (intermediate versus high risk). When assessing DNA methylation impact on prognosis we found a correlation between higher DNA methylation (cumulative levels and numbers of HM genes, respectively) and increased overall survival (OS) (P=0.03 and P=0.01, respectively) as well as lower incidence of relapse (P=0.05 and P=0.01, respectively). For individual genes, *SOCS1* was found to be significantly linked with better OS (P=0.04). **Conclusions.** Our results show that numbers of simultaneously hypermethylated genes and DNA methylation levels of selected TSG as well as HOX genes differs between AML patients with wild-type and mutated *DNMT3A*. Increased DNA methylation of these TSG is linked with better prognosis in AML patients.This study is a part of the COST Action BM0801 (EuGESMA) and is supported by the Czech Ministry of Education, Youth and Sports (MŠMT) OC10042 grant.

0040

TEL2/ETV7 IS A COOPERATING FACTOR IN THE LEUKEMOGENIC TRANSFORMATION OF MN1-TEL T(12;22)(P13:Q11) KNOCK-IN MURINE MYELOID CELLS *IN VITRO*

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The MN1-TEL fusion oncoprotein in human myeloid leukemia is a product of t(12;22)(p13;q11) chromosomal translocation and consist of N-terminal MN1 and C-terminal of TEL1/ETV6 sequences. Previously, our studies have shown that MN1-TEL knock-in mice, in which the *MN1-TEL* transgene is under the control of the *Aml1* regulatory sequences, developed T-lymphoid tumors with 30% penetrance after a long latency period. Furthermore, 90% of mice receiving transplants of Hoxa9-transduced MN1-TEL knock-in BM succumbed to acute myeloid leukemia much more rapidly than mice receiving transplants of control Hoxa9-transduced BM. These results indicated that the leukemogenic effect of MN1-TEL in our knock-in mice is pleiotropic and the cooperating secondary mutation determines the type of leukemic disease outcome. *TEL2/ETV7* is highly homologous to the ETS transcription factor *TEL1/ETV6*, a frequent target of chromosomal translocation in human leukemia. We have shown that TEL2 cooperates with Myc in B lymphomagenesis and ectopic expression of TEL2 in mouse bone marrow causes myeloproliferative disease with a long latency, indicating that TEL2 is a hematopoietic oncoprotein requiring cooperating mutations to induce leukemic transformation. However, the molecular mechanism of TEL2-mediated leukemogenesis in myeloid cells remains to be clarified. *TEL2* expression is upregulated in 70% of ALL or AML samples and 3 out of 3 leukemic MN1-TEL BM samples showed a 2-16 fold upregulation of *TEL2* expression compared with normal BM. Thus, we wished to determine if elevated TEL2 expression cooperated in the leukemogenic transformation in our MN1-TEL mouse model. Here, we focus on the tumorigenic function of TEL2 in context of MN1-TEL knock-in murine bone marrow cells *in vitro*. To induce elevated TEL2 expression, we transduced lineage-negativeBM cells of MN1-TEL$^{+/-}$ mice with lentiviruses encoding *TKp-TEL2-IRES-YFP* or *TKp-IRES-YFP*, which were then cultured in medium supplemented with myeloid cytokines (mIL3, hIL-6 and mSCF). After long-term culture, TEL2-expressing myeloid cells doubled 2-fold faster than control cells in an IL-3-dependent manner. Annexin V staining showed that the rate of apoptosis in TEL2-myeloid cells was 5-fold lower than in control cells. Moreover, TEL2-myeloid cells were more resistant to cytokines withdrawal-induced cell death than control cells. Using quantitative RT-PCR, the expression level of *Bcl-2* was slightly but significantly elevated in TEL2-myeloid cells. In silico analysis identified a highly conserved putative ETS-binding site in *Bcl-2* promoter region. ChIP assay with anti-TEL2 antibody using U937T-TEL2 human monocyte cell line showed that TEL2 specifically bound to the conserved ETS binding site in the human *Bcl-2* promoter region, suggesting that TEL2 directly upregulates *Bcl-2* expression in myeloid cells. These results suggest that TEL2-mediated upregulation of Bcl-2 in MN1-TEL-expressing myeloid cells causes inhibition of apoptosis and might convey a proliferative advantage to MN1-TEL-positive AML cells.

0041

MK-2206 EFFECTIVELY TARGET LEUKEMIA CELLS VIA GSK3-BETA MEDIATED MCL-1 MODULATION

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Background. Phosphoinositide 3-kinases (PI3Ks)/Akt cascade has been found to regulate cell survival, proliferation, and differentiation in a variety of cells. Taking-over upstream signaling from receptor tyrosine kinases (RTKs) or G protein-coupled receptors (GPCRs), activated PI3K further transfers signals to downstream components such as PDK1 and Akt constructing a classical PI3K/AKt pathway. Recent comprehensive genomic analyses of cancers have linked genetic mutations or alterations of critical components in PI3K pathway together with several human cancers. This pathway therefore appears an opportunity for cancer therapy target. PI3K pathway aberration has also been found in patients with acute myeloid leukemia (AML), implicating as a causal

factor in leukemogenesis and drug resistance in AML. **Aims.** Akt makes up one of the major nodes in PI3K pathway so that it has been an important target for specific inhibitors developed by many pharmaceutical companies and academic laboratories. The purpose of this study was to characterize the therapeutic potency and antitumor mechanisms of MK-2206, a specific allosteric Akt inhibitor, in AML. **Methods.** Human leukemia cell lines U937, OCI/AML3, MV-4-11 as well as MOLM-13 and primary AML blast cells from patient were used as *in vitro* and *ex vivo* models for this study. MTS assay was used to determine the effect of MK-2206 on cell viability in AML cells. Western blotting, flow cytometry and quantitative real-time PCR were applied to clarify molecular mechanisms of MK-2206 in AML cells. **Results.** We found that MK-2206 exerted more cytotoxic effect to AML cells in comparison with normal PBMC (IC_{50}: 0.6-2.5 μM vs. 18.8-19.5 μM) suggesting a drug potency with biosecurity. Treatment of MK-2206 results in dose-dependent cell cycle arrest at G1 phase and/or apoptosis induction in all AML cells we tested and primary AML blasts. Further analysis of pro- or anti-apoptotic proteins after MK-2206 treatment revealed that MK-2206 exerts a fast and lasting effect on down-regulation of Mcl-1 in all AML cells we tested. Further elucidating the mechanism how to modulate Mcl-1 expression was explored. We found that MK-2206 treatment did not affect Mcl-1 mRNA expression even at 10 μM for 24hrs, completely ruling out the transcriptional regulation of MK-2206 to Mcl-1 in this issue. We also found that MK-2206 could decrease inactive form of GSK3β (p- GSK3β) accompanying with decreased Mcl-1 within 2 hrs after treatment, suggesting a post-translational mechanism involved. MG-132, a proteasome inhibitor, could rescue Mcl-1 down regulated by MK-2206, confirming the effect of MK-2206 associated with proteasome-dependent degradation. Lithium, an inducer of GSK3β phosphorylation, could also rescue Mcl-1 down regulated by MK-2206, demonstrating that the effect of MK-2206 on modulating Mcl-1 levels is through GSK3β signaling. Taken together, these data highlight a mechanism that MK-2206 would inhibit the Mcl-1 accumulation through activating GSK3b activity and enhancing subsequent proteasome-dependent degradation of Mcl-1. **Conclusions.** MK-2206 showed therapeutic potency in AML treatment and this effect is trough a GSK3b-mediated proteasome-dependent pathway. These data highlight the anti-tumor mechanisms of MK-2206 in AML cells and may help appropriate application of MK-2206 to future clinical trials in AML.

0042

COMPLEX MOLECULAR REARRANGEMENTS IN CHILDHOOD ACUTE MYELOGENEOUS LEUKEMIA WITH TRANSLOCATION T(10;11)(P12;Q23) REVEALED BY PAIRED-END MAPPING

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Background. In up to 20% of all pediatric AML cases rearrangements involving the *MLL* gene on chromosome 11q23 have been reported. Reciprocal translocations t(10;11)(p12;q23) and other more complex rearrangements in 10p and 11q have been detected in 10-15% of pediatric patients with MLL rearranged AML. This cohort depicts a subgroup of AML in children with a very poor clinical outcome with a 5 year overall survival less than 30%. In most cases complex rearrangements with three or more breaks result in a fusion gene consisting of MLL and MLLT10. **Aims.** In this study we report on sequencing results of six pediatric AML patients with variant forms of t(10;11). Paired-end sequencing was performed to give a detailed genetic "map"of the leukemic samples in order to characterize the malignancy and detect further structural variants (translocations, deletions and inversions). **Methods.** DNA was isolated from peripheral blood lymphocytes and paired-end sequencing was performed on an Illumina platform. Patient fragment libraries with insert sizes of ~ 350 bp were sequenced on the GAIIx (2x 36 bp) or the HiSeq 2000 (2x 50 bp), respectively. Reads were aligned using BWA and unique reads with high mapping quality served as input for the structural variation detection tool GASV. Only variants not appearing in the remission sample or the Database of Genomic Variants were reported for closer inspection. Furthermore copy number variations were detected with the tool FREEC. **Results.** The GAIIx produced 96.000.000 to 287.000.000 total reads per sample (2-6 lanes on one flow cell), the HiSeq2000 201.000.000 to 670.000.000 total reads per sample (1-2 lanes on one flow cell). In each sample >90% of reads could be aligned and >90% of the genome was covered by fragments. In five out of six patients MLLT10/MLL was detected and the breakpoint region was identified. In the sixth patient FISH revealed MLLT10/MLL; paired-end-sequencing detected another translocation t(10;11) instead, resulting in a fusion gene consisting of RNF169 and MLLT10. In selected samples, PCR amplification and subsequent capillary sequencing could validate these results. Furthermore, we detected numerous additional structural variants. False positive variants could be reduced after having established an in-house database. Copy number analysis was performed to detect gains and losses of chromosomal regions; in addition, we identified the genetic background of marker chromosomes and were able to visualize complex rearrangements (see figure) .**Summary and Conclusions.** By means of paired-end sequencing we were able to characterize complex genomic structural variants in pediatric patients suffering from t(10;11) AML. Newly detected structural variants in our patients are subject to further analyses in other cohorts to find pathogenic similarities between different entities and characterize prognostic subgroups.

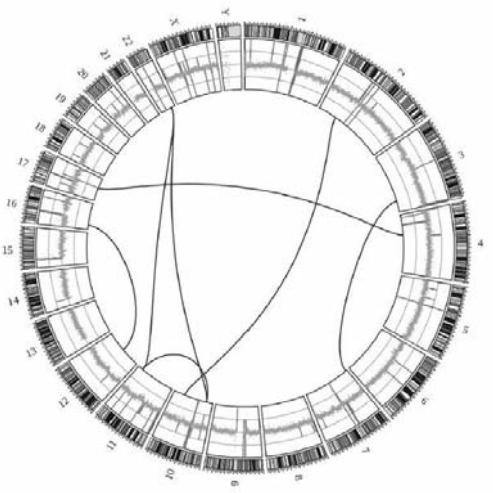

Figure 1. CIRCOS Plot for circular genome data visualization.

0043

A HIGH FREQUENCY OF GENE MUTATIONS INVOLVING EPIGENETIC REGULATORS IN MINIMALLY DIFFERENTIATED ACUTE MYELOID LEUKEMIA

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Background and Aims. Minimally differentiated acute myeloid leukemia (AML-M0) is a rare subtype of AML with poor prognosis. Novel genetic aberrations are increasingly reported in AML but have not been studied comprehensively in AML-M0. We aimed to examine the genetic alternations in patients with AML-M0 and to determine their clinical relevance. **Materials and Methods.** Sixty-eight patients diagnosed as *de novo* AML-M0 with median age of 49.0 (0.3~97.9) and median follow-up duration of 4.6 (0~176.6) months were enrolled. We analyzed a wide spectrum of gene mutations including class I of signaling and RAS pathways (*FLT3*, *C-FMS*, *C-KIT*, *N-RAS*, *K-RAS*, *PTPN11*, *JAK2*^{V617F}), class II affecting transcription and differentiation (*RUNX1*, *MLL*-PTD, *NPM1*, *CEBPα)*, class III of tumor suppressor genes (*WT1*, *P53*), and class IV of epigenetic regulators (*IDH1/2*, *ASXL1*, *DNMT3A)* on bone marrow cells from AML-M0 patients at initial diagnosis. Gene mutational analysis was performed with PCR-based assays followed by direct sequencing. **Results.** Fifty-three of 68 (77.9%) patients were found to have at least one mutation: 50.8% (33/65) patients had class I mutations which were mutually exclusive, 33.3% (22/66) had class II, 9.2% (6/65) had class III and 35.4% (23/65) had class IV mutations. The most frequent gene mutations were *FLT3* (28.4%, 15 *FLT3*-ITD and 4 *FLT3*-TKD), *IDH1/2* (27.7%, 4 *IDH1*-R132, 5 *IDH2*-R140, 9 *IDH2*-R172), *RUNX1* (23.9%), *N-RAS/K-RAS* (12.3%), *ASXL1* (10.9%), *DNMT3A* (10.3%) and *MLL*-PTD (9.1%). *PTPN11*, *WT1*, *P53*, *C-FMS*, *JAK2*^{V617F}, *NPM1*, and *CEBPα* occurred sporadically with lower frequencies. Taken together, 26 of 53 patients (49.1%) had more than one mutation. Four of the 6 patients with *DNMT3A* and 3 of the 7 patients with *ASXL1* mutations also had *IDH2* mutations. *IDH* and *RUNX1* mutations were associated with older age (p=0.006 and p=0.011). *RAS* mutation was associated with higher circulating blasts whereas patients with *DNMT3A* mutations had a significantly lower WBC counts and lower circulating blasts. In univariate analysis, survival was affected by old age (HR 1.019, 95% CI 1.009~1.029, p=<0.001), cytogenetic risk group (intermediate vs. high risk, HR 0.486, 95% CI 0.237~0.996, p=0.049) and the presence of class III mutations (HR 1.512, 95% CI 0.985~2.321, p=0.059). Old age (HR 1.014, p=0.005) and class III mutations (HR 1.638, p=0.026) had adverse impact on event-free survival. In multivariate

analysis, old age (HR 1.021, 95% CI 1.008~1.034, *p*=0.001) and *RUNX1* mutation (HR 2.712, 95% CI 1.241~5.930, *p*=0.012) remained the adverse factor for inferior survival. **Conclusions.** The present study showed a high frequency of class IV mutations (*IDH1/2, ASXL1* and *DNMT3A,* single or in combination*)* in AML-M0 in addition to *FLT3* and *RUNX1* mutations. Most patients harbored multiple mutations. *RUNX1* mutation was associated with a poor outcome. Supported by grants NSC100-2314-B-182-023-MY3, NHRI-EX96-9434SI and MMH-E-99009.

0044

KPT-SINE (SELECTIVE INHIBITORS OF CRM1 MEDIATED NUCLEAR EXPORT) EXHIBIT STRIKING ANTI-LEUKEMIC ACTIVITY AGAINST AML AND T-ALL CELLS WHILE SPARING NORMAL HEMATOPOIETIC CELLS

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Background. CRM1 is the major nuclear exporter that mediates transport of a variety of molecules, including proteins involved in tumor suppressor and cellular proliferation pathways. CRM1 is upregulated in a range of solid and hematologic malignancies and its overexpression is correlated with poor prognosis and with resistance to chemotherapy. The crystal structure of CRM1, in complex with its export cargo, Snurportin 1, has been recently resolved. The molecular understanding of the CRM1-cargo binding interface has led to the development of novel small molecule inhibitors of CRM1-cargo interaction, termed KPT-Selective Inhibitors of Nuclear Export (SINE). **Aims.** KPT-SINE are potent CRM1 inhibitors that irreversibly inactivate the CRM1-directed protein export by covalent modification of the essential CRM1-cargo binding residue Cys528. The inhibition of the CRM1 nuclear export has been shown to lead to selective apoptosis in cancer cells when compared to normal hematopoietic cells and normal cells. Here, we assess the efficacy of the KPT-SINE in human AML, T-ALL, and normal hematopoietic cells. **Methods.** The viability of a panel of human AML and T-ALL cell lines upon treatment by the KPT-SINE was assessed. Dose-response measurements were done using serial dilutions of KPT-SINE from 1 μM to 0.3 nM and luminescent cell viability assay. Apoptosis was measured using Annexin V staining and TUNEL assays. **Results.** KPT-SINE induces rapid apoptosis in 12 AML and 14 T-ALL cell lines with IC50s of 15-170 nM. In the KPT-SINE-sensitive cell lines, BCL2 overexpression suppresses KPT-SINE-induced apoptosis, indicating its intrinsic pathway mediation. Importantly, oral administration of KPT-SINE compounds, either KPT-251 at 75 mg/kg or KPT-330 at 15 or 25 mg/kg, induced remarkable growth suppression in MV4-11 human AML cells engrafted into immunodeficient NSG mice with minimal toxicity to normal mouse hematopoietic cells after 35 days of treatment. Bone marrow biopsies of KPT-treated mice were remarkable in that they showed normal hematopoietic cell morphology and cellularity after 35 days of treatment. Significant survival benefit was observed in mice treated with either KPT-251 or KPT-330, compared to vehicle-treated mice. Studies to address the potency of the KPT-251 and KPT-330 on MOLT-4 human T-ALL cells *in vivo* are ongoing. **Conclusions.** These studies emphasize the clinical promise of the KPT-SINE as a novel and selective drug candidate for the treatment of AML and T-ALL.

0045

VECTORIZED ANTICANCER DRUG F14152, USED ALONE OR IN COMBINATION WITH ARAC, DEMONSTRATES POTENT ANTILEUKEMIC ACTIVITY IN AML *in vivo* MODELS DERIVED FROM PATIENTS

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Background. Drugs specifically vectorized to cancer cells should offer a reinforced activity, tackle tumor cells while preserving normal cells, resulting in an improved therapeutic index. In this context, the selective targeting of tumor cells with polyamine-containing drugs has been considered and the most promising compound in this category is arguably the spermine-podophyllotoxin conjugate F14512. F14512, that contains a spermine chain in place of the C4 glycosidic moiety of etoposide, displays an enhanced anti-proliferative activity on a large panel of tumor cell lines (10 fold) as compared to etoposide and exploits the polyamines transport system (PTS) to accumulate into cells. In a previous study, we established a clear correlation between the expression of the PTS system in 13 leukemia cell lines and their sensitivity to the cytotoxic action of F14512. This antitumor activity has encouraged the set up of a phase 1 clinical study with F14512 in patients with acute myeloid leukemia (AML). **Aim.** Although standard cytotoxic chemotherapy induced complete remissions in AML patients, relapse rates are still very high, resulting in a poor outcome in most cases. **Methods.** We evaluated the antitumor activity of F14512 alone, and in combination with the reference drug AraC, against novel *in vivo* AML models, collected from 3 different patients, and established onto NSG mice (LAM-2, LAM-7 and LAM-18). **Results.** These 3 AML samples exhibited a normal karyotype, with FLT3-ITD, NPM1 and DNMTA3 mutations which proved their stability over serial transplantations *in vivo*. After multiple i.v. administrations of F14512, 3 times a week for 3 weeks, an extensive reduction of AML cell number (98-99%) was observed in LAM-2 and LAM-7 -bearing mice. This antileukemic activity was recorded on the basis of flow cytometry, q-PCR and histology assessments. Mechanisms of F14512-induced cell death are currently investigated, and preliminary data suggest that senescence is involved. We also demonstrated *in vitro* and *in vivo* synergistic effects of F14512 in combination with AraC, one of the frontline chemotherapeutic agents for AML. The antileukemic effects of F14512 on LAM-18 bearing mice were marginal with an inhibition of AML cell growth of 42%. **Summary and Conclusions.** Collectively, these results demonstrate that F14512 exhibits a marked *in vivo* antileukemic activity in these AML models derived from patients, supporting its clinical development, which is currently in progress.

0046

A SPECIFIC MICRORNA SIGNATURE CHARACTERIZES ACUTE MYELOID LEUKEMIA WITH TRANSLOCATION T(6;9) (P23;Q34)/DEK-NUP214

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Background. Acute myeloid leukemia (AML) with translocation t(6;9)(p23;q34) is a rare (1%) high-risk AML subtype characterized by the fusion of nuclear phosphoprotein DEK in chromosome 6 with the nucleoporin encoding gene *NUP214* (*CAN*) in chromosome 9. This fusion gene in der(6) chromosome induces transformation by altering growth characteristics, nucleo-cytoplasmic transport, and protein synthesis of hematopoietic stem cells. MicroRNAs (miRNAs) are small non-coding RNAs that play an important regulatory role in many biological processes. Nonetheless, to date, little is known on the role of miRNAs in t(6;9) (p23;q34) DEK-NUP214 AML subtype [t(6;9) AML]. **Aims.** The main objective of this study was to examine if t(6;9) AML harbors a miRNA signature associated with DEK/CAN fusion gene assembly. **Methods.** We have analyzed the miRNA profile of 117 AML patients with 10 different AML subtypes, including eight cases of t(6;9) AML, and three CD34+ bone marrow specimens from healthy donors. The expression of 670 mature miRNAs was assessed using TaqMan Human MicroRNA Arrays v2.0 (Applied Biosystems) in an ABI 7900 HT sequence detection system. Statistical analyses were performed with TiGR MultiExperiment Viewer and R software. To identify molecular pathways potentially altered by the expression of multiple miRNAs we used Diana-mirPath, which performs an enrichment analysis of multiple miRNA target genes, comparing each set of miRNA targets to all known KEGG pathways. **Results.** Supervised analysis by means of t-test based on multiplex permutations and SAM analysis revealed a distinctive miRNA signature in t(6;9) AML patients. When the miRNA expression in t(6;9) AML was compared with miRNA expression in all other AML subtypes and also specifically with other high-risk AML subtypes, we found that a miRNA signature specifically associated with t(6;9) AML. This specific signature is composed of 30 differentially expressed miRNAs, 28 of which are upregulated in comparison with other leukemia subtypes. Some of these miRNAs have been previously described as players in several cancers (let-7a and let-7e, miR-143, miR-145, miR-451, miR-330-5p, miR-194, miR-99, miR-132, miR-199, miR-494 or miR-98), and others have been related to acute leukemia (miR-223, miR-451,miRNA-181a*). In addition, several HOX-related miRNAs were upregulated (miR-10a, miR-10a* and miR-10b). Interestingly, three miRNAs of the signature are located in or near the genomic region of NUP214 (9q34): miR-181a* (9q33), let-7a (9q34) and miR-199b-5p (9q34). The Diana-mir-Path analysis found that some of the miRNAs in our signature may regulate genes involved in the KIT, MAPK/AKT2, and RUNX1 signaling pathways, both of which are deregulated in AML.**Conclusions**We have identified for the first time a specific miRNA signature related to t(6;9) AML, with an overall upregulated profile. Some of the miRNAs in the signature may impact the leukemogenic process by altering essential pathways involved in cell differentiation and proliferation. Our findings thus contribute some insight into the biological profile of t(6;9) AML. Nonetheless, further investigation is warranted to determine the mechanisms leading to this miRNA signature and to identify the specific targets of the miRNAs.

0047

IDENTIFICATION OF TARGET GENES OF THE TRANSCRIPTION FACTOR HB9 IN HEMATOPOIETIC CELLS AND ITS ROLE IN HEMATOPOIESIS

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Background. The transcription factor HB9, encoded by the *homeobox gene B9* (*HLXB9*), is involved in the development of pancreatic beta- and motor neuronal cells. In addition, *HLXB9* is recurrently rearranged in young children with Acute Myeloid Leukemia (AML) characterized by a chromosomal translocation t(7;12)-*HLXB9/TEL* and concomitant high expression of the unrearranged, wildtype *HLXB9* allele.Hence in this study we aimed to identify target genes of HB9 in hematopoietic cells and its role in hematopoiesis. **Methods and Results.** In this study, we used ChIP-on-chip analysis together with expression profiling and identified prostaglandin E receptor 2 (PTGER2) as a direct target gene of HB9 in a hematopoietic cell line. The functional HB9 homeodomain is essential for binding of HB9 to the *PTGER2* promoter region and enables downregulation of *PTGER*2 expression. Functionally, HB9 conducted downregulation of *PTGER2* results in a two-fold repression of intracellular cAMP mobilisation when cells were stimulated with butaprost and a four-fold decrease when stimulated with prostaglandin E2. The decreased gene expression is valid in *HLXB9* expressing primary murine hematopoietic stem cells as well as in bone marrow cells from translocation t(7;12) positive patients. Furthermore, among the primary and secondary target genes of HB9 in the myeloid cell line HL60, 78% of significantly regulated genes are downregulated, indicating an overall repressive function of HB9. Differentially regulated genes were preferentially confined to pathways involved in cell-adhesion and cell-cell interactions, similar to the gene expression footprint of *HLXB9* expressing cells from t(7;12) positive patients. **Conclusions.** The present results indicate a regulative function of HB9 in cell-adhesion and cell-cell interaction in hematopoietic cells. Future experiments will analyse the role of *HLXB9* in the regulation of bone marrow homeostasis using an in-vivo model of primary murine HSCs and a transgenic Nestin-GFP positive mouse strain to model the hematopoietic niche.

0048

CLINICAL IMPLEMENTATION OF A NOVEL HIGH-THROUGHPUT SCREEN OF PRIMARY LEUKEMIA CELLS TO PERSONALIZE THERAPY FOR RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA (AML)

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Background. For most patients with relapsed and refractory acute myeloid leukemia, therapeutic success is unpredictable with current cytotoxic chemotherapeutic regimens. Moreover, multiple courses of therapy have been shown to result in co-morbid conditions, which can often preclude the patient from being able to receive the potentially curative therapy of allogeneic bone marrow transplantation. **Aims.** With the goal of identifying a successful chemotherapy regimen for these patients, based on patient-specific myeloid blast cell chemo-sensitivities, we have developed a high-throughput screening assay that utilizes primary (patient-derived) leukemia cells and tested for chemo-sensitivity and resistance against a panel of established chemotherapeutic agents as well as novel agents. **Methods.** This assay was developed in 1536 well format and initially optimized against a panel of leukemia and lymphoma cell lines to help determine optimal concentration of cells to be used. Standard twelve point dose response curves were performed in duplicate for all compounds tested. Alamar Blue was used as an indicator of cell viability and death. **Results.** Using this assay with primary patient samples of AML, we were able to identify *in vitro* resistance against the various drugs already clinically administered, including cytarabine, etoposide, and anthracyclines. Importantly, we were also able to show *in vitro* sensitivity to chemotherapy agents that had not been previously administered. The index patient was a 32-year-old woman with primary refractory acute myeloid leukemia who had received six different therapeutic regimens prior to our testing, all of which demonstrated *in vitro* resistance using our assay. The blasts were sensitive, however, to 6-thioguanine with an inhibitory concentration (IC_{50}) of 70 nM. Based on the *in vitro* data, the patient began a combination treatment regimen containing oral 6-thioguanine as her white blood cell (WBC) count had increased to greater then 50,000 cells/ml on her previous therapy. Following a maintenance regimen with these agents, her circulating blast count decreased to less then 10,000 cells/ml, and she had partial recovery of the neutrophil count, resulting in a dramatic decrease in the overall leukemia burden. This result was not seen with her previous treatment regimens. Importantly, the majority of this therapy was administered in the outpatient setting. After over six months of combination therapy, her WBC began to rise again and repeat testing was performed on her cells. Importantly, the results indicated resistance to 6-thioguanine with IC_{50}>10 microM, confirming the response seen *in vivo*. Subsequently, we have collected samples from more than fifteen patients with AML and used this screening assay to predict their response to various chemotherapeutics. In each case, we have accurately predicted the *in vivo* clinical response (both sensitivity and resistance) to different chemotherapeutic agents. **Conclusions.** These data demonstrate that the technology described here to screen drug therapy for patients with relapsed and refractory AML is clinically useful and has a potential role in designing individualized drug treatment regimens for these patients.

0049

DIFFERENTIAL EXPRESSION OF NPM1 SPLICE VARIANTS IN ACUTE MYELOID LEUKEMIA

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Background. Acute myeloid leukemia (AML) is one of the most common types of leukemia among adults. Recently, gene mutations and deregulated expression of genes have been described, providing insights into the mechanisms of leukemogenesis and unraveling the enormous molecular heterogeneity, in particular within the group of cytogenetically normal (CN) AML. Of particular importance are mutations of the necleophosmin-1 (*NPM1*) gene, which identify a group of CN-AML patients with favorable prognosis in case of no concomitant FMS-like tyrosine kinase 3-internal tandem duplication (*FLT3*-ITD). Notably, wildtype *NPM1* encodes for three alternatively spliced isoforms: R1 (B23.1), R2 (B23.2), R3 (B23.3). **Aims.** Since splicing variants play an important role in cellular function, the current project aimed to characterize the expression pattern of *NPM1* splice variants in AML patients. **Methods.** For 96 AML patient samples (44 CN-AML and 52 samples with cytogenetic aberrations) qRT-PCR was performed. Total expression (Rt) as well as levels of the three splicing variants of *NPM1* were evaluated: R1 translates exon 1 to 9 and 11 to 12, R2 contains exons 1 to 10 and R3 lacks exons 8 and 10. To investigate the distribution of splicing variants the R1/Rt, R2/Rt and R3/Rt ratios were calculated, respectively.

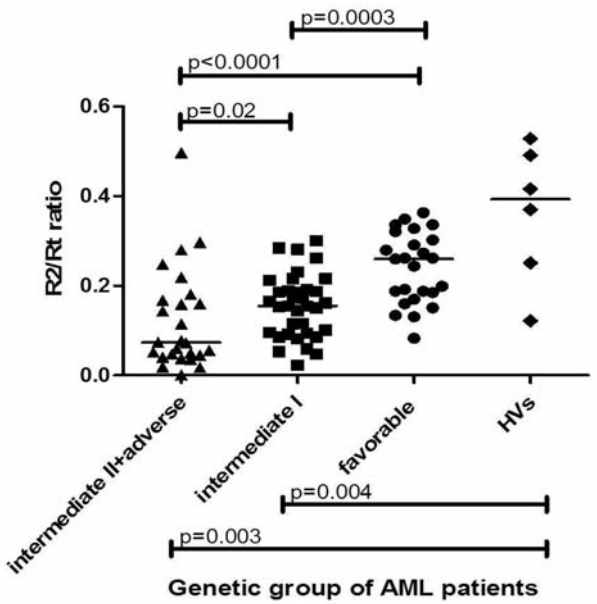

Figure 1. Differential expression of R2/Rt ratio in the total AML cohort with different prognosis. Favorable (t(8;21), inv(16), *NPM1+/FLT3*-ITD-), intermediate I (*NPM1+/FLT3*-ITD+, *NPM1-/FLT3*-ITD-, *NPM1-/FLT3*-ITD+), intermediate II and adverse (t(9;11), complex karyotype, cytogenetic abnormalities not classified as favorable or adverse).

Results. Total expression Rt as well as expression of splice variants R1 and R3 were significantly higher in all AML patients compared to healthy volunteers (HVs) with a median expression of 1.323 vs 0.5175 (p=0.004), 2.98 vs 0.5485

(p=0.0072), and 2.821 vs 0.108 (p<0.0001), respectively. The expression of R2 splicing variant was elevated in AML patients with *NPM1* mutations (*NPM1*+) compared to patients without *NPM1* mutations (*NPM1*-) with a median expression of 0.829 vs 0.385 (p=0.0426). Significant differences with regard to the splicing variant distribution were found between HVs, CN-favorable (*NPM1*+/*FLT3*-ITD) and CN-intermediate (*NPM1*+/*FLT3*-ITD+; *NPM1*-/*FLT3*-IDT-; *NPM1*-/*FLT3*-ITD+) groups. The ratios of R1/Rt and R2/Rt within the CN-intermediate group were significantly lower in comparison to HVs (median 0.231 vs 0.48, p=0.0048 and 0.155 vs 0.393, p=0.0038, respectively), while the R3/Rt ratio was increased in CN-intermediate and CN-favorable groups compared to HVs (median 0.617 vs 0.084, p= 0.0002 and 0.3555 vs 0.084, p=0.0007). Interestingly, in all AML patients the R2/Rt ratio was the highest in groups with favorable prognosis and was decreased in groups with poor outcome (Figure 1). **Conclusions.** *NPM1* mutations play an important role in the biology of the CN-AML bearing both prognostic and predictive value. Interestingly, the expression and the distribution of *NPM1* splice variants might be of biological importance for the entire AML cohort, as we found an imbalance in the distribution among all AML patients, not only CN-AML. Notably, there was a great reduction of a *NPM1* splice variant R2 that lacks exons 11 and 12 (R2/Rt), where the mutation appears.

0050

THE LEUKEMIA ASSOCIATED NUCLEAR CO-REPRESSOR ETO HOMOLOGUE GENES ARE REGULATED DIFFERENTLY IN HEMATOPOIETIC CELLS

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Backbround. *ETO, MTG16,* and *MTGR1* are nuclear transcriptional corepressors of the human ETO protein family. The ETO homologue genes are 3´participants in leukemia fusions generated by chromosomal translocations responsible of hematopoietic dysregulation. **Aims.** We tried to compare structural and functional promoter elements of *ETO*, *MTG16* and *MTGR1* genes in order to find associations between their regulation and hematopoiesis. **Methods.** 5´ deletion examinations and luciferase reporter gene studies were used to identify the proximal promoter. Binding of transcrition factor to the TFBS site was confiemed by *in vitro* antibody enhanced electrophoretic moblity shoft assay and *in vivo* chromatin immunoprecipitation. Overexpression study was used to confrim the effect of transcription factor on expression of genes. **Results.** An evolutionary conserved region of 228 bp revealed potential cis-elements involved in transcription of ETO. While, TATA- and CCAAT-less MTG16 promoter with a GC box close to the start site showed strong reporter activity when examined in erythroid/megakaryocytic cells. Mutation studies showed that an evolutionary conserved GATA consensus-binding site present at -636 and -301 site of MTG16 and MTGR1 promoter respectively, repressed promoter function of MTG16 and MTGR1. Furthermore, results from *in vitro* antibody-enhanced electrophoretic mobility shift assay and *in vivo* chromatin immunoprecipitation validated the binding of GATA-1 to the GATA site. The overexpresion of GATA1 futher increased the activity of *ETO* and *MTGR1* promoer in reporter assay. In case of MTGR1, TATA-less and CCAAT-less promoter retained most of the transcriptional activity in a GC box-rich sequence containing multiple SP1 binding sites reminiscent of a housekeeping gene with constitutive expression. However, mutations of individual SP1 binding sites did not repress promoter function; multiple active SP1 binding sites may safeguard constitutive *MTGR1* transcriptional activity. The leukemia associated *AML1-ETO* fusion gene suppressed *ETO, MTG16* and *MTGR1* promoter activity. **Conclusions.** An evolutionary conserved GATA binding site is critical in transcriptional regulation of the *ETO* and *MTG16* promoter. In contrast, the *MTGR1* gene depends on a GC box-rich sequence for transcriptional regulation and ubiquitous expression. Our results demonstrate that the ETO homologue promoters are regulated differently consistent with hematopoietic cell-type-specific expression and function.

Acute myeloid leukemia - Clinical 1

0051

ADDITION OF MINIMAL RESIDUAL DISEASE (MRD) ASSESSMENT BY FLOW CYTOMETRY FOR FURTHER RISK STRATIFICATION WITHIN THE EUROPEAN LEUKEMIA NET FAVORABLE RISK GROUP IN ADULT AML

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Background. In adult AML, most patients receiving intensive induction chemotherapy achieve complete hematologic remission. However, the majority of these patients will experience subsequent relapse. Therefore, determination of relapse risk is of particular interest to guide therapeutic decisions for post-remission therapy. To date, cytogenetic and molecular markers are able to provide risk stratification at primary diagnosis. However, the value of MRD is emerging and might play an increasing role in risk stratification beyond genetics. MRD assessment by flow cytometry is possible in virtually all cases, even those where molecular markers are not available.AimsIn order to further improve existing and well described risk stratification, we analyzed whether the addition of MRD-assessment by flow cytometry after induction chemotherapy in patients achieving complete remission is capable of determining groups of patients which, within each cytogenetic and molecular risk group, might have an elevated risk of relapse. **Methods**. From the database at the Laboratory of Leukemia Diagnostics at our clinic patients with newly diagnosed AML treated between 2000 and 2011 were analyzed. At least two samples of bone marrow blood (at primary diagnosis and during or after treatment) had to be available for MRD assessment by 3-color-flow cytometry at our laboratory. Leukemia associated immunophenotypes (LAIPs) were determined by gating on aberrant expression of a well-defined set of antigens (Kern et al., 2003). MRD positivity was defined as ≥0,3% LAIP positive cells after induction therapy. Cytogenetic and molecular risk stratification was assigned in accordance to the European LeukemiaNet (ELN) guidelines. **Results**. 662 patients fulfilled the inclusion criteria and 472 (71%) achieved a complete remission. 346 (73%) of these patients had MRD data by flow cytometry available after induction chemotherapy and in 319 cases ELN risk stratification was available. The median age at diagnosis in this group was 53 years (18-85) and 94, 86, 95 and 44 patients were assigned to the ELN favorable, intermediate I, intermediate II and adverse risk group, respectively. For the entire cohort, LAIP positivity resulted in a significantly shorter Relapse Free Survival (RFS) (median 12,4 vs. 19,9 months, log-rank test p=0,016), whereas Overall Survival (OS) was not significantly different (48,9 vs. 38,1 months, p=0,132). However, within the favorable risk group both RFS (78,2 vs. 20 months, p=0,016) and OS (median not reached vs. 49,6 months, p=0,019) were significantly worse in case of MRD positivity. **Summary.** In adult AML, therapeutic decisions guiding post-remission treatment strategies are mostly based on molecular and cytogenetic risk at primary diagnosis. Here we could show that MRD assessment by flow cytometry after induction therapy can further divide the ELN favorable risk group into two distinct subgroups. This might lead to improved risk stratification to guide post-remission therapeutic decisions. However, the value of MRD assessment needs to be confirmed in prospective studies.

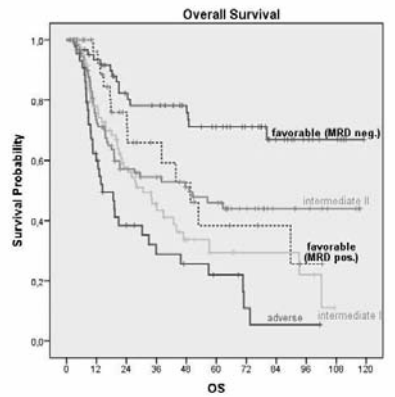

Figure 1. Overall survival showing subgroups within the ELN favorable risk group by MRD status (log rank test p<0.00001)

0052

MICRORNA LET-7A-3 PROMOTER METHYLATION IS ASSOCIATED WITH KARYOTYPING, CEBPA PROMOTER METHYLATION AND SURVIVAL IN ACUTE MYELOID LEUKEMIA

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Background. Epigenetic dysregulation plays an important role in oncogenesis. In acute myeloid leukemia (AML), a number of genes, such as *CDKN2B (p15), p73, SOCS1* and *CEBPA,* are aberrantly methylated. MicroRNAs (miRNAs) are endogenous small non-coding RNAs. They target the 3' untranslated regions of certain mRNAs and repress their expression post-transcriptionally. The miRNA let-7a is involved in oncogenesis by targeting growth-control genes. It is transcribed from three different loci, the *let-7a-1*, *let-7a-2*, and *let-7a-3*, where *let-7a-3* is located in a CpG island and is epigenetically regulated. *Let-7a-3* is differentially methylated in normal and tumor tissues, and hypermethylation of *let-7a-3* is correlated with favorable prognosis in ovarian cancer. However, the significance of epigenetic phenotype of *let-7a-3* in AML has not been addressed. **Aims.** We examined the methylation status of *let-7a-3* promoter in AML cell lines and patients and evaluated the association of the methylation level to clinical features and survival of AML patients. **Methods.** A total of 90 patients diagnosed with AML were enrolled in this study. This study was approved by the Institutional Review Board of the NTUH, and written informed consents were obtained from all the participants. Bisulfite-treated DNA was used in the DNA methylation analysis which was designed to cover a 723-bp region containing 33 CpG sites including two *Bst*UI sites (CGCG) in the *let-7a-3* gene. DNA methylation status was assessed by combined bisulfite restriction analysis (COBRA) and bisulfite sequencing PCR (BSSP). **Results.** We showed that *let-7a-3* promoter was heavily methylated in AML cell lines and most of the AML patients. *Let-7a-3* was methylated at 93%, 80%, 71%, and 90% of the CpG sites in AML cell lines including HEL, HL60, K562 and U937 cells, respectively. *Let-7a-3* was designated as methylated in 81.1% (73/90) patients, as partially methylated in 12.2% (11/90) patients, and as unmethylated in 6.7% (6/90) patients. To evaluate the clinical significance of *let-7a-3* methylation, chi-square test was applied to correlate the methylation status and clinical data of 84 AML patients. *Let-7a-3* methylation status was correlated with patients' karyotypes but not with age, gender, and FAB subtypes. In patients with favorable karyotypes [t(15;17), t(8;21), and inv(16)], 40% (4/10) acquired hypermethylated *let-7a-3*, whereas in patients with intermediate (normal and others) and unfavorable (-5, -7, +8 and complex) karyotypes, the frequency was 83% (49/59) and 86% (13/15), respectively. The transcription factor C/EBPα is a tumor suppressor essential for early myeloid differentiation. Mutation and epigenetic silencing of *CEBPA* are both linked to AML development. We found that methylation status of *let-7a-3* was inversely correlated with methylation of *CEBPA* distal promoter region (-1423 to -1121), which is crucial for *CEBPA* expression. In patients with unmethylated *CEBPA*, 88% (56/64) harbored hypermethylated *let-7a-3*; however, in patients with hypermethylated *CEBPA*, only 50% (10/20) did. Using Kaplan-Meier survival analysis, we demonstrated that *let-7a-3* hypermethylation was associated to better 5-year survival in AML patients with intermediate karyotype or unmethylated *CEBPA*. **Summary and Conclusions.** Our study proposed a prognostic role for *let-7a-3* methylation in AML and indicated an inverse correlation between the methylation status of *let-7a-3* and *CEBPA*.

0053

DETERMINATION OF THE MAXIMUM TOLERATED DOSE OF PANOBINOSTAT IN COMBINATION WITH A 5-DAY SCHEDULE OF AZACITIDINE IN HIGH RISK MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKAEMIA: A PHASE IB/II STUDY

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Background. The therapeutic options for patients with high risk myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) who are not eligible for intensive chemotherapy remain limited. The combination of hypomethylating agent and deacetylase inhibitor (DACi) has been shown to be synergistic, both in terms of leukemia cell killing and gene reactivation *in vitro*. **Aims.** To investigate the safety, tolerability and preliminary efficacy of combining the oral pan-DACi panobinostat (LBH589) with azacitidine in newly diagnosed MDS or AML, not fit for standard induction therapy. **Methods.** Phase Ib/II multi-center open label dose-escalation and expansion study. Inclusion criteria: untreated IPSS intermediate-2 or high risk MDS, or AML (marrow blasts ≥20%), not eligible for standard induction therapy. Patients received azacitidine 75 mg/m^2 SC on days 1-5 of each 28-day cycle with either 10, 20, 30 or 40mg panobinostat orally 3 days per week (M/W/F) for 7 doses per cycle commencing on day 5. Treatment schedule contained 6 cycles of combination therapy, and continued if there was no disease progression or unacceptable toxicity. The safety and tolerability of the combination was assessed. **Results.** 40 patients gave informed consent and were enrolled into the study, with a median age of 70 years (36-82). 30 AML patients had intermediate (18/30) or poor cytogenetic risk (12/30); 10 MDS patients with intermediate-2 (7/10) or high risk (3/10) IPSS. Patients entered panobinostat dose-escalation cohorts of 10mg (4 patients), 20mg (7), 30 mg (6) or 40mg (6); and expansion study 30mg (17). All grade non-hematologic adverse events regardless of relatedness to study treatment (>10%) were fatigue (50%), injection site reaction (50%), nausea (38%), diarrhea (33%), anorexia (28%), febrile neutropenia (15%), constipation (13%), dyspnea (13%), fever (13%), and vomiting (13%). There were no unexpected adverse events or drug reactions. The principal dose-limiting toxicity (DLT) was fatigue. In the dose-escalation phase, the grade 3/4 DLTs were: panobinostat 10mg cohort (0/4 DLT), panobinostat 20mg cohort (1/7 DLT; grade 3 fatigue), panobinostat 30 mg cohort (1/6 DLT; grade 3 fatigue), panobinostat 40mg (4/6 DLTs; all grade 3: fatigue (1), syncope (1), hyponatremia (1) and somnolence/reduced level of consciousness (1)). Therefore, in combination with the 5-day schedule of azacitidine, the maximum tolerated dose (MTD) of panobinostat was defined at 30mg; this dose level was selected for expanded accrual. The median number of treatment cycles initiated was 5 (1-20). There was an overall response rate (ORR) (CR+CRi+PR) in AML patients of 8/30 (27%) (2 CR, 1 CRi, 5 PR). In MDS patients the ORR was 5/10 (50%) (2 CR, 3 PR). After a median follow-up of 10.6 months, the median OS was 8.0 months (0.7-20.5). **Conclusions.** In previously untreated MDS/AML, panobinostat and azacitidine is well tolerated and the MTD of panobinostat was 30mg when in combination with a 5-day azacitidine schedule at 75mg/m^2 daily. This combination demonstrates clinical activity and warrants further clinical evaluation.

0054

AZACITIDINE IS WELL TOLERATED AFTER ALLOGENEIC STEM CELL TRANSPLANTATION AND ITS ADMINISTRATION IS ASSOCIATED WITH A REDUCED RISK OF ACUTE GRAFT-VERSUS-HOST DISEASE

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Background. Graft-versus-host disease (GVHD) and disease relapse are the major causes of treatment failure in adults with Acute Myeloid Leukaemia (AML) who undergo a reduced intensity conditioned (RIC) allograft. Strategies which reduce the risk of disease relapse and GVHD are urgently required. The demethylating agent 5-azacitidine (AZA) has well documented anti-leukaemic activity in AML and has been shown to reduce the risk of GVHD in animal models. **Aims.** We wished to study the tolerability of post-transplant AZA and its impact on GVHD and disease relapse in patients undergoing a RIC allograft for high risk AML. **Methods.** Adults with AML who underwent allogeneic transplantation using a conditioning regimen consisting of fludarabine (30 mg/m^2 IV x 5 days), melphalan (140 mg/m^2 IV), and alemtuzumab (10 mg IV x 5 days) were eligible for this study. All patients received GVHD prophylaxis using cyclosporine. Patients with sustained neutrophil and platelet engraftment commenced treatment with AZA (36 mg/m^2 x 5 days) which was administered every 28 days until one year post transplant. All patients gave informed consent and the trial was registered as #ISRCTN36825171. **Results.** 37 patients (median age 59 yrs) commenced treatment with AZA at a median of 55 days (range 40 to 194) post-transplant. 25 patients were transplanted from matched (7 or 8/8) unrelated donors and 12 patients were transplanted from HLA identical related donors. 32 patients were in remission at the time of transplant (CR1=24, CR2=8) and 5 patients had active disease (2 primary refractory AML, 3 relapsed AML). 21 patients had intermediate risk cytogenetics and 9 poor risk cytogenetics at diagnosis. Post transplant AZA was well tolerated and 31 patients completed at least 3 cycles. Haematological toxicities were modest and only 4 patients experienced treatment delay due to neutropenia or thrombocytopenia. The most common grade >3 non-haematological toxicity was infectious complications which occurred in 9 patients.

5 patients developed Grade 2 acute GVHD and no patient developed >Grade 2 acute GVHD. 3 patients developed limited chronic GvHD and no patient developed chronic extensive GVHD. To date 11 patients have relapsed at a median of 7 months (4-15 months) after transplantation. Of the 16 patients treated with AZA until one year post transplant, 15 are in complete remission at the time of last follow up. **Conclusions.** Prolonged administration of AZA is well tolerated immediately after a RIC allograft in an elderly population of patients. Our data demonstrate that administration of AZA after allogeneic SCT appears to be associated with a low risk of acute and chronic GVHD and a reduced risk of disease relapse. These observations identify a potential strategy to improve outcome after allogeneic stem cell transplantation in high risk AML but require confirmation in a randomised clinical trial.

0055

HIGH GATA2 EXPRESSION IS A POOR PROGNOSTIC MARKER IN PEDIATRIC ACUTE MYELOID LEUKEMIA

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Background.Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease that accounts for 15% to 20% of childhood leukemia. Over the past decades, the prognosis for children with AML has improved considerably as a result of better (risk-adapted) therapeutic strategies. Risk-group classification has mainly been based on cytogenetic aberrations and response to induction chemotherapy. More recently, novel genetic defects and expression patterns have been discovered that enable the further refinement of risk-category assignment for individual patients. **Aims.** The transcription factor GATA binding protein-2 *(GATA2*) gene, located at chromosome 3q21, plays an essential role in the regulation of myeloid lineage determination. In the current study we investigated the relevance and prognostic value of *GATA2* expression and mutations in pediatric AML. **Methods.** In a large cohort of *de novo* pediatric AML patients, mutation screening of *GATA2* was performed using Sanger sequencing. *GATA2* expression was determined using gene expression profiling (Affymetrix HGU133 Plus 2.0 microarray) and quantitative real-time PCR. Informed consent was obtained after Institutional Review Board approval according to local laws and regulations, and in accordance with the Declaration of Helsinki.Results*GATA2* mutations were detected in 5/230 pediatric AML cases, representing a frequency of 2.2% overall, and 9.8% in cytogenetically normal (CN-)AML. Co-occurrence of *GATA2* mutations with various other recurrent mutations was observed in all cases (*N-RAS* mutations (N=4), *NPM1* mutations (N=2), *CEBPA* double mutations (N=2), and *WT1* mutations (N=1)). The clinical significance of *GATA2* mutations should be determined in a larger cohort. Both at diagnosis and clinical relapse, *GATA2* expression was heterogeneous. In 151 out of 237 patients (64%), *GATA2* expression at diagnosis was higher than in normal bone marrow. In a subgroup of 38 patients, *GATA2* expression was studied during follow-up. In complete remission, normalization of *GATA2* expression was observed, whereas *GATA2* expression levels stayed high in patients with refractory disease.Importantly, high *GATA2* expression at diagnosis proved to be an independent poor prognostic factor for event free survival (HR 2.1, p=0.001) and disease free survival (HR 2.2, p=0.005), with a trend towards worse overall survival (HR 1.7, p=0.056). The prognostic impact of *GATA2* was particularly high in specific morphologic and genetic AML-subgroups. High *GATA2* expression identified a group of patients with a significantly worse outcome among patients with high *WT1* expression. Moreover, in patients with FAB-M5 morphology and inv(16), pronounced differences in survival were observed between patients with high vs. normal *GATA2* expression. **Summary and Conclusions. .** We conclude that high *GATA2* expression is a novel poor prognostic marker in pediatric AML, which identifies patients with a severely dismal prognosis in specific AML subgroups. Since *GATA2* was a potent poor prognostic factor for overall survival, event free survival and disease free survival, *GATA2* expression may be a useful marker for better risk-group stratification and risk adapted therapy in the future.

0056

SALVAGE THERAPY WITH HIGH-DOSE CYTARABINE AND MITOXANTRONE IN COMBINATION WITH ALL TRANS RETINOIC ACID AND GEMTUZUMAB OZOGAMICIN IN PRIMARY REFRACTORY ACUTE MYELOID LEUKEMIA

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Background. The prognosis of patients with acute myeloid leukemia (AML) refractory to first induction therapy is poor and highly dependent on response to salvage therapy as well as subsequent allogeneic hematopoietic stem cell transplantation (HSCT). Best outcome can be achieved after allogeneic HSCT performed in complete remission (CR). Therefore, improvement of response to salvage therapy is mandatory. **Aims.** The primary goal of the AMLSG 05-04 trial of the German Austrian AML Study group (NCT00143975; ClinicalTrials.gov) was to assess the response rate and overall survival (OS) in patients < 61 years of age with primary refractory AML treated with high-dose cytarabine and mitoxantrone in combination with all-*trans* retinoic acid (ATRA) and gemtuzumab ozogamicin (GO).Here we report the final results including long-term survival data. **Methods.** Patients with AML refractory to first induction were eligible for enrollment. All patients gave informed consent.Salvage therapy (GO-A-HAM) consisted of GO 3mg/m^2, iv on day 1; cytarabine 3mg/m^2, iv bid. days 1-3; mitoxantrone 12mg/m^2, iv days 2 and 3 and ATRA 45mg/m^2, po days 4-6 and 15mg/m^2 po days 7-28.In all patients an allogeneic HSCT was intended after completion of salvage therapy. **Results.** 93 patients were enrolled between July 2004 and June 2007. All patients received prior intensive induction therapy with idarubicin, cytarabine and etoposide. Median age at enrollment was 48 years (range: 22-62), type of AML was *de novo* n=77; secondary, n=4, and therapy-related n=12. Distribution of cytogenetic risk groups revealed n=7 (8%) favorable risk, n=51 (58%) intermediate risk, and n=30 (34%) high risk patients. *FLT3*-ITD and *NPM1* mutations were present in n=18 (22%) and n=12 (15%) patients, respectively. CD33 (>20% expression measured by flow cytometry) was expressed on leukemic blasts in 87% of the patients.Results after GO-A-HAM were as follows: CR was achieved in n=28 (30%), CR with incomplete platelet recovery (CRi) in n=19 (20%), partial remission (PR) in n=10 (11%), refractory disease (RD) in n=33 (35%), and n=3 patients (3%) died. Major toxicities CTC > grade 2 were infections (65%), gastrointestinal symptoms (14%), and neurological impairments (8%). Seventy (75%) patients proceeded to an allogeneic HSCT (n=16, MRD; n=49, MUD; n=5, haplo-identical donor) all within 3 months after GO exposure. Nine cases of veno-occlusive disease (VOD) after HSCT occurred, 5 (7%) of them were graded moderate/severe.Median follow-up was 48.6 months, 4-year OS rate was 32% (95%-CI 24%- 44%). Best OS rate was achieved in patients attaining CR, CRi or PR after GO-A-HAM who received subsequent allogeneic HSCT (n=50) with 47% (95%-CI, 35-64%). In contrast, OS rate was poor in patients without response to GO-A-HAM and subsequent allogeneic HSCT (n=20) or those not receiving an allogeneic HSCT irrespective of response to GO-A-HAM (n=23) with 12% each. (95%-CI 3-45%; 4-42%)No prognostic marker for response to salvage therapy or survival was identified. **Conclusions.** GO-A-HAM is an effective treatment option for primary refractory AML patients leading to high response rates. The combination with subsequent allogeneic HSCT was feasible without increased VOD rate. Survival of responding patients after allogeneic HSCT was promising.

0057

LOW-DOSE LENALIDOMIDE IN ADDITION TO LOW-DOSE CYTARABINE INDUCES LONG-LASTING COMPLETE REMISSIONS IN A SUBSTANTIAL PROPORTION OF VERY ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS WITH UNFAVORABLE KARYOTYPE

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Aims. We designed a phase II study to assess the antitumor efficacy of the combination regimen with low-dose lenalidomide and low-dose cytarabine in patients with acute myeloid leukemia (AML) aged more than 70 years. **Methods.** Thirty-three patients (median age 75 years, range: 70-85) were consecutively enrolled in the study. Median white blood cell count at diagnosis was $5.5x10^9$/l (range: 0,59-46,$8x10^9$/l), whereas median haemoglobin was 9,4 g/dl and median platelet count was $29x10^9$/l. Ten out of 33 patients had a normal karyotype, 19/33 an intermediate or unfavourable karyotype and 4/33 were not evaluable. Sixteen patients had a *de novo* AML, whereas 17 patients had a secondary AML (12 after MDS, 2 after a CMPD, 1 after myelofibrosis, 2 after chemo-radiotherapy for a breast cancer). Patients received low-dose lenalidomide (10 mg/day orally, days 1-21) and low-dose cytarabine (20mg twice day subcutaneously, days 1-15). Therapy was repeated every 6 weeks, up to 6 cycles. **Results.** Seven out of 33 patients died in aplasia while receiving the first induction cycle of therapy, and are not evaluable for response; one is not evaluable due to death while receiving chemotherapy (acute heart failure). Four patients are too early. Twenty-two patients completed at least one cycle of therapy and are evaluable for response. Among these patients, 11/22 (50%) obtained complete remission (CR) after the first cycle, recovering normal WBC, hemoglobin and platelets values after a median of 32 days (range: 25-44) from the start of chemotherapy. Eight out of 11 responding patients are still in morphologic, cytogenetic and FISH CR after 24, 22, 20,14, 11, 10, 5 and 3 months from the start of therapy, respectively. Two patients died while in CR after receiving, respectively, the second and the third cycle of therapy due to a multi organ failure after an infectious complication. One patient relapsed 3 months after the first cycle and died with active leukemia. The other 11 patients who completed at least one cycle of therapy did not respond at all and rapidly died due to progressive disease. At present, with a median follow up of 13 months for surviving patients, 8/22 (36%) are alive in continuous CR, 2/22 died in CR and 1/22 died due to relapse. Notably, all responding patients presented with low blast count and unfavorable cytogenetics at diagnosis. **Conclusions.** Low-dose lenalidomide has high activity in addition to low-dose cytarabine, in a subset of very elderly AML patients with extremely poor-prognosis. Considering the low compliance of very elderly, frail AML patients to high-dose therapy, this low dose schedule seems to be particularly profitable for patients with low blast count and unfavorable cytogenetics. The study was registered at EMA with the EUDRACT no 2008-006790-33.**Acknowledgements:** Celgene is acknowledged for providing Lenalidomide for the patients. The study was supported in part by AIL Pesaro Onlus.

0058

HIGH BONE MARROW EXPRESSION OF LGALS3, THE GENE ENCODING GALECTIN-3, IS AN INDEPENDENT UNFAVORABLE PROGNOSTIC FACTOR FOR OVERALL SURVIVAL IN PATIENTS WITH *de novo* ACUTE MYELOID LEUKEMIA

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Background. Galectin-3, a β-galactoside-binding lectin, plays an important role in cancer cell progression, adhesion, apoptosis, transformation, angiogenesis and metastasis. A lot of researches also demonstrated an important role of galectin-3 expression in several types of malignancies. However, the studies concerning clinical implications of galectin-3 expression in patients with acute myeloid leukemia (AML) are scarce. **Aims.** In this study we aimed to determine the clinical implication of the expression of *LGALS3,* the gene encoding galectin-3, in adult *de novo* non-M3 AML patients. **Methods.** We investigated the expression of *LGALS3* in the bone marrow (BM) by reverse-transcriptase real-time polymerase chain reaction in a test cohort consisting of 280 adults 15 years of age or older with newly diagnosed *de novo* non-M3 AML at the National Taiwan University Hospital and correlated the results with clinical features and outcomes of the patients. The prognostic impact of BM *LGALS3* expression was also validated in an independent cohort comprised 42 *de novo* non-M3 AML patients. **Results.** Among 280 patients, those with higher BM *LGALS3* expression were older (P<0.001), more frequently had French-American-British (FAB) M4 and M5 subtypes (both P=0.003), and CD14 expression on leukemic cells (P=0.009), but less commonly had FAB M1 subtype (P<0.001). In addition, higher *LGALS3* expression was closely associated with *PTPN11* mutation but negatively associated with *FLT3-ITD* and *CEBPA*$^{\text{double mutation}}$. There was no correlation between the cytogenetic abnormalities and BM *LGALS3* expression. Survival analysis was performed in 211 non-M3 AML patients who received standard intensive cure-intent chemotherapy. Patients with higher BM *LGALS3* expression, compared to those with lower expression, had lower CR rates (61.5% *vs.* 82.5%, P=0.001) and higher primary refractory rates (23.1% *vs.* 10.8%, P=0.023). With a median follow-up time of 69.5 months, patients with higher BM *LGALS3* expression had a shorter overall survival than those with lower expression (median 16.3 months *vs*. 39.8 months, P=0.02). Moreover, multivariate analysis demonstrated that higher BM *LGALS3* expression was an independent poor prognostic factor for overall survival among total patients and patients with normal karyotype. The unfavorable prognostic impact of higher BM *LGALS3* expression was also confirmed in the independent validation cohort. A scoring system incorporating high BM *LGALS3* expression and nine other prognostic factors, including age, white blood cell count, cytogenetics, *NPM1/FLT3-ITD*, *MLL-PTD*, *CEBPA*$^{\text{double mutation}}$ and mutations of *AML1/RUNX1* and *WT1*, into survival analysis was proved to be very useful to stratify AML patients into different prognostic groups (P<0.001). **Summary and Conclusions.** Our research provides evidences that BM *LGALS3* expression is associated with distinct biologic and clinical characteristics in AML. Higher BM *LGALS3* expression is significantly correlated with poor prognosis in AML patients. BM *LGALS3* expression may serve as a new biomarker to predict clinical outcome. Galectin-3 may be a potential target for the treatment of AML patients with higher expression of this factor.

0059

THE INCIDENCE OF CENTRAL NERVOUS SYSTEM INVOLVEMENT IN ACUTE MYELOID LEUKEMIA

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Background. Acute myeloid leukemia (AML) is able to affect the central nervous system (CNS). However, so far most studies examined paediatric patients or reported about small numbers of patients. Thus, little is known regarding the clinical aspects and treatment outcome of CNS-involvement in adult AML. **Aims.** To analyze the clinical appearance, risk factors and impact of CNS involvement in a large series of adult patients with AML treated within the studies of the Study Alliance Leukemia (SAL) at initial diagnosis or relapse. **Methods.** 3530 AML patients with a median age of 57 years (range 15-87) included in the prospective AML96, AML2003 and AML60+ trials of the SAL study group were evaluated for CNS involvement. Patients with neurological symptoms were referred to an aspiration of the cerebrospinal fluid (CSF) and CNS involvement was proven depending on morphology and/or flow cytometry of the CSF. Prophylactic CNS therapy was not regularly performed within these treatment protocols. CNS involvement was treated using intrathecal triple chemotherapy (ITC). **Results.** A total of 50 of 3530 patients had proven CNS involvement with an overall incidence of 1.4%. Patients with CNS involvement were similar to patients without CNS involvement with respect to gender, age, *FLT3/NPM1* mutations and AML-therapy.The incidence of extramedullary AML other than CNS was significantly higher in patients with CNS involvement (n=26, 52 % *vs.* n=246, 7%, *p<0.001*). AML FAB M5 occurred more frequently in patients with CNS involvement (n=16, 32% *vs.* n=452, 13%, *p<0.01*). Furthermore, patients with CNS involvement had significantly higher lactate dehydrogenase (LDH) levels (1049 *vs.* 421 IE/l, *p<0.001*), higher white blood cell count (WBC) (64 *vs.* 11 Gpt/l, *p<0.001*) and had a more often trisomy 8 (n= 8, 16% *vs.* n= 277, 8%, *p=0.03*). Comparing patients with CNS involvement with patients without CNS involvement at diagnosis no significant difference in overall survival at 2 years (40% *vs.* 41%) and event free survival at 2 years (20% *vs.* 26%, respectively) was observed.20 patients had CNS involvement at time of initial presentation and 30 patients had CNS involvement at first, second or third relapse. Only 6 of 20 patients (30%) with CNS involvement at initial diagnosis developed systemic relapse, similar to patients without CNS involvement (n=1243, 35%). Furthermore, none of the patients in this group developed CNS involvement at the time of relapse. On the contrary all patients with CNS involvement at relapse developed again systemic, as well as CNS relapse (n=30,100%). **Conclusions.** CNS involvement in AML is a rare entity and it is accompanied with higher incidence of other sites of extramedullary AML. Factors associated with an increased risk of CNS involvement include: trisomy 8, FAB M5, higher LDH levels and higher WBC count at diagnosis. Patients with CNS disease at initial diagnosis of AML have similar survival outcomes as compared to those without CNS disease if treated with intrathecal therapy, whereas extramedullary relapse within the CNS is associated with a poor outcome.

0060

POSITIVE PROGNOSTIC IMPACT OF > 4 LOG REDUCTION OF NPM VALUE AFTER INDUCTION AND CONFIRMED MOLECULAR REMISSION IN DENOVO CYTOGENETICALLY NORMAL AML PATIENTS WITH NPM MUTATIONS

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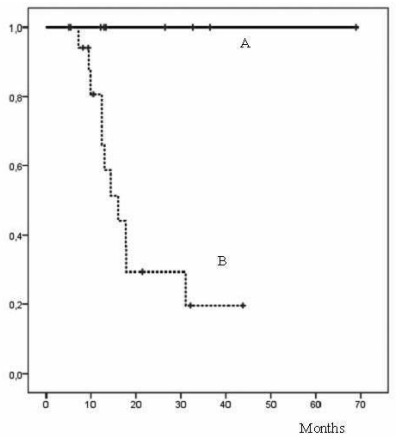
Background and Aims. In the subset of NPM-mutated denovo AML patients (NPM-AML), the study of NPM mutations has been proposed as a means to assess response to therapy and monitor minimal residual disease (MRD). We compared the clinical value of MRD monitoring by cytofluorimetric methods, WT1 expression and by the study of NPM mutations in a series of NPM-AML patients. **Patients and Methods.** Bone marrow samples of 26 consecutive normal karyotype NPM-AML patients (median age 54 years) achieving CR after conventional chemotherapy were studied. A four-color flow cytometer (FCM) was used to perform immunophenotype (IF). NPM A mutations and WT1 expression were studied by a quantitative Real Time PCR. Immunophenotypic complete response (IF-CR) was defined as the absence of clonal cells by FCM. Lack of NPM mutation was defined as NPM molecular complete response (NPM-CR), whereas the normalization of WT1 expression defined WT1 molecular complete response (WT1-CR). If the undetectability of the NPM mutation was confirmed in two consecutive samples, the response was defined as confirmed NPM-CR. **Results.** The relapse risk was not affected by the achievement of IF-CR and WT1-CR after consolidation therapy, but it was significantly lower in patients achieving a confirmed NPM-CR (3/10 in confirmed NPM mol-CR and 15/16 in patients without confirmed NPM mol-CR, p<0.03). The projected DFS of patients achieving a < 4 log or ≥ 4 log reduction in NPM values after induction therapy were 12.6% and 50%, respectively at 36 months (p=0.009). Moreover, DFS was clearly affected by the achievement of a confirmed NPM-CR (36 month-projected DFS is 63% for patients who reached confirmed NPM-CR and 0% for those who did not, p 0.001). Cox regression analysis showed that DFS was influenced only by the achievement of confirmed NPM-CR (p 0.004) and by blast count at diagnosis (p 0.003). Furthermore, FLT3-ITD status at diagnosis had no impact on DFS in the cohort of patients reaching NPM-CR or in those with confirmed NPM-CR. The projected OS of patients achieving a < 4 log or ≥ 4 log reduction of NPM values after induction therapy was 19.6% and 100%, respectively, at 36 months (p = 0.01) (Figure 1). The reappearance of clonal cell at FCM analysis (IF-rel), WT1-molecular relapse (WT1 rel) and NPM-molecular relapse (NPM rel) were always followed by hematologic relapse (H-rel). The median interval between IF-rel, WT1-rel and NPM-rel and H-rel was 0.8 months (range 0-4.6), 1.2 months (range 0-4.7) and 4.4 months (range 0.9-8.4), respectively. The interval between NPM-rel and H-rel was significantly longer than the interval between IF-rel and H-rel (p<0.01) or WT1-rel and H-rel (p<0.03). **Conclusions.** In NPM-AML DFS and OS were mainly affected by the kinetics of the clearance of NPM mutated cells (> 4 log reduction or NPM-CR after induction) and by the achievement of confirmed NPM-CR after consolidation. Furthermore studying NPM mutations in the follow up period allows for early prediction of H-rel compared to FCM and evaluation of WT1 expression.



A: survival of patients with ? 4 log NPM reduction after induction

B: survival of patients with < 4 log NPM reduction after induction

Figure 1. OS according to NPM log reduction after induction.

0061

PROLONGED THROMBOCYTOPENIA IN ACUTE MYELOID LEUKAEMIA (AML) PATIENTS TREATED WITH FRACTIONATED DOSES OF GEMTUZUMAB OZOGAMICIN (GO) IN THE ALFA-0701 STUDY DOES NOT CONFER AN ADVERSE PROGNOSIS

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Aim. Previous studies with conventional doses of GO have reported prolonged thrombocytopenia as a frequent adverse event. The ALFA-0701 trial demonstrated the efficacy of adding 5 doses of 3 mg/m2 GO on day 1, 4 and 7 of a standard 7+3 induction and day 1 of two consolidation courses in patients with *de novo* AML aged 50-70 years (median, 64y) (Castaigne et al., ASH meeting 2011). However, despite the use of fractionated small doses prolonged thrombocytopenia were more frequent in the GO arm B than in control arm A. We report here on their incidence and prognostic significance. **Methods.** According to IWG criteria, complete remission with incomplete platelet recovery (CRp) is defined as CR with a platelet count (PLTC) <100.109/L. Prolonged thrombocytopenia was defined here as no PLTC >100.109/L recovery on day 45 following the onset of previous chemotherapy course (grade 1-2, PLTC >50 and <100.10.9/L; grade 3-4, PLTC ≤ 50.109/L). **Results.** The CR/CRp rate was 104/139 (74.8%) in arm A and 113/139 (81.3%) in GO arm B. (P=0.25), with more CRp in arm B (11 vs 4). During induction, 3 patients in arm A and 4 in arm B died with haemorrhage. Among CR/CRp patients, 86.5% in arm A and 76.1% in arm B received the 2 planned consolidation courses (P= 0.07). The reason for not receiving consolidation therapy was prolonged thrombocytopenia in 8 arm B patients. Overall, 19 (14 grade 1-2 and 5 grade 3-4) and 74 (53 grade 1-2 and 21 grade 3-4) episodes of prolonged thrombocytopenia were observed in 14 arm A and 48 arm B patients, respectively (P<0.0001), either after induction (4 arm A and 12 arm B), first (7 arm A and 28 arm B), or second consolidation (8 arm A and 34 arm B). Thrombocytopenia resolved within 2 to 29 months (median, 5 months) in 4/19 and 34/74 episodes in arm A and B, respectively (P= 0.07). In the other cases, thrombocytopenia was still present at the time of relapse, death, or transplant. Median number of platelet transfusion episodes was significantly higher in arm B after the 3 chemotherapy courses. One CRp patient died with haemorrhage in arm B. Grade 3-4 bleeding events occurred in 4/139 (2.9%) in arm A and 12/139 (8.6%) in arm B (P= 0.07). In arm B, RFS was similar in CR and CRp patients (2-year RFS, 50.1% [40.3-62.2] vs 51.9% [28.7-93.9]; P=0.88). Based on univariable analyses in the 217 responders, persistent thrombocytopenia was only associated with liver toxicity (44% in patients with liver toxicity vs 25% of those without, P= 0.03). Among the 208 responders alive at 6 months, 57 (11 arm A, 46 arm B) had previously experienced prolonged thrombocytopenia, but this did not modify their survival (2-year OS, 71.7% vs 53.2%.; HR= 0.63 [0.35-1.13]; P= 0.12) and was even associated with an improved RFS (2-year RFS, 51.2% vs 31.5%; HR= 0.61 [0.39-0.96]; P= 0.03). **Conclusions.** prolonged thrombocytopenia, even if frequently observed after GO treatment, does not confer an adverse prognosis.

0062

CD56 ANTIGEN EXPRESSION AS THE MOST IMPORTANT PROGNOSTIC FACTOR IN NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA WITHOUT THE FLT3-ITD MUTATION

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Background. Patients with normal karyotype acute myeloid leukemia (NK-AML) without the FLT3 internal tandem duplication (FLT3-ITD) mutation account

for approximately 30% of all AML cases and exhibit a heterogeneous clinical outcome. The prognostic factors in this subgroup of AML patients are still unknown. **Aims.** The aims of this study were to estimate the prognostic factors for complete remission (CR) rate, overall survival (OS) and disease-free survival (DFS) in patients with NK-AML in the absence of the FLT3-ITD mutation as well as to determine the relevance of CD56 antigen expression, an immunophenotypic marker, on outcome in this cohort of patients. **Methods.** The study involved 112 subjects with NK-AML without the FLT3-ITD mutation during a follow-up period of 42 months. As risk factors for rate of CR, OS and DFS in months in this cohort of patients the following were evaluated: age, ECOG performance status (PS), leukocytosis (<30x10^9/L vs ≥3010^9/L), lactate dehydrogenase (LDH) more than 1.5 x upper limit of normal, and CD34 expression (<10% vs ≥10%). For CD56 antigen expression, detected by flow-cytometry, the cut-off value ≥20% was taken as positive (CD56+). Patients were treated by the Medical Research Council (MRC) 12 regimen. Risk factors were identified using univariate and multivariate analysis. **Results.** The mean age of the patients was 55 years (range 21-79). Univariate analysis showed that the following risk factors were significant for CR rate: age ≥55 years (p = 0.012), ECOG PS ≥2 (p = 0.011), leukocytosis (p = 0.030), LDH (p = 0.011), and CD56+ (p = 0.025). Multivariate analysis indicated CD56+ as the most important risk factor for CR rate: p = 0.005, HR 0.266 (95%CI 0.086-0.765). Significant factors for OS in univariate analysis were: age ≥55 years (p = 0.013), ECOG PS ≥2 (p = 0.020), leukocytosis (p = 0.046), LDH (p = 0.050) and CD56+ (p = 0.024). Multivariate analysis indicated CD56+ as the most important risk factor for OS: p = 0.008, HR 2.180 (95%CI 1.352-3.962). CD56+ was not significant for DFS in this subset of patients. **Conclusions.** This study showed that CD56 antigen expression on leukemic cells in patients with NK-AML without the FLT3-ITD mutation predicts shorter OS and a lower rate of CR in comparison with patients from the same subgroup without CD56 positivity. Therefore, more intensive therapeutic regimens should be considered in such cases.

0063

THE ROLE OF MAINTENANCE THERAPY IN ACUTE PROMYELOCYTIC LEUKEMIA - A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background. Acute promyelocytic leukemia (APL) is the most curable type of AML, with a cure rate over 80%. A consensus exists regarding the administration of both induction and consolidation treatments, albeit different approaches. However, there is conflicting evidence regarding the effect of maintenance treatment on survival. **Aims.** Objectives of this review were to examine the efficacy and safety of maintenance therapy in patients with APL and to establish the best regimen for maintenance in these patients. **Methods.** We performed a systematic review and meta-analysisof randomized controlled trials assessing maintenance treatment in patients with newly diagnosed APL in first complete remission. We searched *The CochraneLibrary*, MEDLINE, EMBASE, LILACS, conference proceedings, databases of ongoing trials, and references of published trials until September 2011. As different comparator arms were used during maintenance, we pooled data for the following comparisons: any maintenance *vs.* observation; ATRA-based maintenance *vs.* non- ATRA based maintenance and ATRA alone *vs.* ATRA and chemotherapy. Primary outcomes were overall survival (OS) and disease-free survival (DFS). Hazard ratios (HR) with 95% confidence intervals (CI) were estimated and pooled using random-effects model. **Results.** Nine randomized controlled trials enrolling 2012 patients were included in the meta-analysis. According to the three above designed comparisons, there is no evidence that maintenance therapy improves OS (HR for any maintenance *vs*. observation 1.07, 95% CI 0.23-4.86; HR for ATRA-based maintenance *vs*. non- ATRA based maintenance 0.98, 95% CI 0.64-1.51; HR for ATRA alone maintenance *vs*. ATRA and chemotherapy 1.13, 95% CI 0.62-2.07). However, DFS was improved with any maintenance compared to observation (HR 0.63, 95% CI 0.41-0.97); (5 trials, 1209 patients). Moreover, ATRA based regimens improves DFS compared to non-ATRA based regimens HR 0.61 95% CI 0.46-0.83); (4 trials, 749 patients), with further benefit for ATRA and chemotherapy maintenance compared to ATRA alone (HR 1.47 95% CI 1.03-2.1; 4 trials, 1028 patients). The report on major adverse events was scarce, precluding meta-analysis of clinically relevant adverse events. However, increased rates of grade 3/4 adverse events were noted for any maintenance *vs*. observation and for ATRA and chemotherapy *vs*. ATRA alone. **Conclusions.** Results of the present meta-analysis suggest that maintenance therapy improves DFS in APL patients compared to observation and does not support an OS benefit. The significance of this finding is limited due to significant clinical and statistical heterogeneity between studies (I^2 of heterogeneity=72%). ATRA based regimens improve DFS but not OS compared to non-ATRA based regimens, with further benefit for ATRA and chemotherapy compared to ATRA alone. The discrepancy between the advantage in terms of DFS and the lack of influence on OS in all comparisons might be explained by an increased toxicity of maintenance which might negatively balance the improved DFS or by effective salvage protocols for relapsing patients. The effect of maintenance therapy should be further evaluated in the various prognostic groups, and the different induction and consolidation regimens.

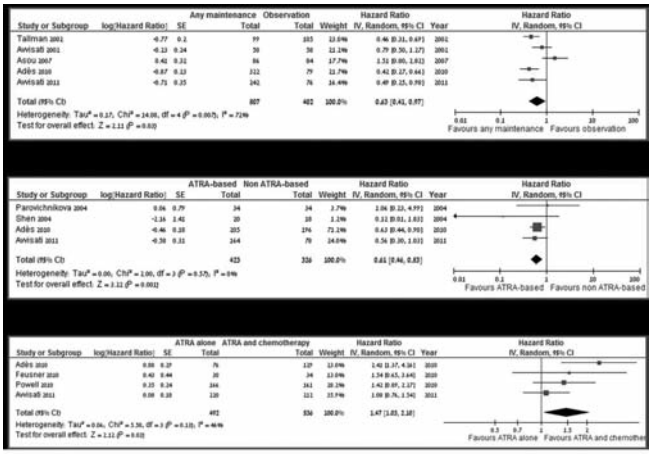

Figure: disease-free survival (DFS) for the 3 designed comparisons. Black squares represent the point estimate, their sizes represent their weight in the pooled analysis, and the horizontal bars represent the 95% CI. The black diamond at the bottom represents the pooled point estimate. ATRA, all-trans retinoic acid; CI, confidence interval; SE standard error.

0064

TIME FROM DIAGNOSIS TO INTENSIVE CHEMOTHERAPY INITIATION MIGHT NOT ADVERSELY IMPACT THE OUTCOME OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Although acute myeloid leukemia (AML) is a therapeutic emergency, initiation of chemotherapy is sometimes delayed because of medical, geographical or administrative constraints, with unknown consequences. A recent study (Sekeres *et al*., Blood 2009) tended to show that a 5-day or more delay had a negative impact on response rate and overall survival of patients aged 60 or younger. This conclusion is now cited in international guidelines (Döhner *et al*., Blood 2010). **Methods.** We retrospectively studied the impact on outcome of the time from diagnosis to treatment (TDT) in 552 consecutive patients treated with intensive chemotherapy in Toulouse University Hospital between 2000 and 2009 for non-promyelocytic newly diagnosed acute myeloid leukemia. TDT was transformed using restricted cubic spline method to obtain a continuous function. **Results.** The median age of our cohort was 57 years (range, 16-83), with 61% being 60 or younger. Secondary and hyperleukocytic AML (*ie*. WBC > 50 G/L) counted for 20% and 24% respectively. According to the European LeukemiaNet classification 22%, 29%, 26% and 23% of patients were classified in favorable, int-I, int-II and adverse categories. ECOG performance status > 1, tumoral syndrome, leukostasis and infection at diagnosis were found in 13%, 26%, 4% and 13% respectively. The induction chemotherapy was homogeneous since 94% of patients received either daunorubicin (60 mg/m²/d for three days) or idarubicin (8 g/m²/d for five days) in combination with standard doses of cytarabine (100 or 200 mg/m²/d for seven days according to age. The median TDT was seven days (range, 1-73) with 331 (60%) patients having a TDT longer than 5 days. In the 331 patients whose chemotherapy was delayed more than 5 days, the main causes were diagnosis out of Toulouse University Hospital (26%), an awaited karyotype result (12%), diagnosis out of leukemia unit (12%) or infection at diagnosis (8%). 54 (10%) early deaths occurred in our series. TDT was not significantly associated with early death, both in non-adjusted analysis (p=0.06) and after adjustment (p=0.15). CR or CRi was obtained in 399 patients (72%). After adjustment, TDT was not significantly associated with response rate (p=0.1675 for younger patients and WBC ≤ 50 G/L). The median overall survival was 18 months (12 in older and 28 in younger). TDT was associated with overall survival (p=0.047): the non-adjusted risk of death is U-shaped with a nadir to day 6 (Figure 1a). However, after adjustment for age, secondary AML, WBC, ECOG performance

status, ELN risk groups and type of consolidation, TDT was no longer associated with OS (p=0.74) (Figure 1b). TDT did not have an impact on OS regardless of age and WBC. **Conclusions.** We did not find any harmful signal concerning the effect of TDT on OS, early death and response rate, both in younger and older patients with newly diagnosed AML. Since personalized therapies based on particular genetic features are going to be developed, our study suggests that waiting for specialized laboratory tests does not seem unreasonable, except for well-known early complications.

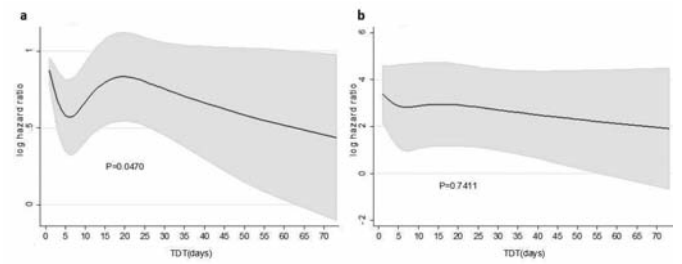

Figure 1.

0065

IDARUBICIN OVERCOMES MULTIDRUG RESISTANT 1 INDUCED CHEMORESISTANCE WITH HIGHER INDUCTION REMISSION RATE THAN DAUNORUBICIN IN *DE NOVO* ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Multidrug resistance 1(MDR1) mediated chemoresistance was extensively considered to play an important role in treatment failure in acute myeloid leukemia(AML). Idarubicin(IDA), a synthetic daunorubicin(DNR) analog, was reported to have a distinct advantage over that of DNR for remission induction combined with cytarabine(Ara-C) in AML. But the underlying molecular mechanisms remained unclear. **Aims.** This study is aiming to determine if IDA can overcome MDR1 induced chemoresistance in *de novo* acute myeloid leukemia patients. **Methods.** Clinical data of 125 patients aged between 12 to 80 years who have received daunorubicin (DNR at 35~45mg/m^2/d, 3 days) or idarubicin (IDA, 6~8 mg/m^2/d, 3 days) in combination with cytarabine (Ara-c 100~200 mg/m^2/d, 7 days) for remission induction were retrospectively analyzed. The patients were subdivided into high and low MDR1 expression groups according to the median expression of MDR1 mRNA in their pre-treated bone marrow cells. Comparisons were performed using X^2 test and independent-samples T test. **Results.** Treatment with IDA and DNR in combination with Ara-C resulted in satisfactory CR rates of 82.5% and 57.4% CR respectively. Patients with high MDR1 expression had a significantly lower CR rate than those with low MDR1 (59.7% vs 77.8%, p=0.029). We then subdivided the patients into IDA/high MDR1, DNR/ highMDR1, IDA/low MDR1, and DNR/low MDR1 groups and compared the CR rate. Results showed CR rate in the IDA/high MDR1 group (23/28, 82.1%) was higher than the DNR/ highMDR1 group (14/34, 41.2%)(P=0.001), while there were no statistical difference between IDA/low MDR1 (24/29, 82.8%)and DNR/low MDR1 group(25/34, 73.5%)(P=0.380), suggesting IDA may overcome MDR1 induced chemoresistance. Besides, the IDA arm was also superior to DNR arm on CR rate in favorable (21/23(91.3%) vs 15/25(60.0%), P=0.012) or intermediate cytogenetic risk groups(22/27(81.5%) vs 20/35(57.1%), P=0.042). To investigate whether MDR1 also has an impact on induction remission in favorable or intermediate risk groups, patients were further divided into high and low MDR1 subgroups in favorable and intermediate risk groups. MDR1 expression level between the two risk groups was not of statistical significance (p=0.645), but CR rate in IDA/favorable/high MDR1 group (9/10, 90.0%) was higher than DNR/favorable/high MDR1 group (4/11, 36.4%) (p=0.011). Similarly, CR rate in IDA/intermediate/high MDR1 group (12/15, 80.0%) was higher than DNR/intermediate /high MDR1 group (8/19, 42.1%)(p=0.026), suggesting overcoming MDR1 induced chemoresistance also potentiated IDA remission induction superiority in favorable or intermediate risk groups. **Conclusions.** IDA was superior to DNR for remission induction with a significantly higher CR rate. Overcoming MDR1 induced chemoresistance and improving the CR rate in favorable and intermediate cytogenetic risk groups potentiated IDA the superiority. A larger prospective clinical trial is needed to further confirm this point of view.

0066

THE CLINICAL FEATURES AND PROGNOSTIC IMPACT OF DNMT3A GENE MUTATION IN JAPANESE PATIENTS WITH *de novo* AML

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Background. and Aims. The *DNA methyltransferase 3A* (*DNMT3A*) gene mutation was identified by whole-genome sequencing in patients with acute myeloid leukemia (AML). *DNMT3A* encodes for the enzyme DNA (cytosine-5)-methyltransferase 3A and belongs to the family of other methyltransferases. These enzymes are involved in adding methyl groups to the cytosine residue of CpG dinucleotides and thus play an important role in epigenetic regulation of genes, but the mechanism of *DNMT3A* mutation -associated leukemogenesis was still unknown. About clinical impact of *DNMT3A* mutation, several groups reported that *DNMT3A* mutation have been associated with adverse outcome. We analyzed clinical features and prognostic impact of *DNMT3A* mutation in patients with Japanese patients with AML. **Methods.** We retrospectively analyzed 188 cases of *de novo* AML treated at Nippon Medical School Hospital and its affiliated facilities from 2000 to 2010. We analyzed 188 samples at initial presentation and 70 samples at relapse. **Results.** *DNMT3A* mutations were detected in 35 cases (18.6%) at initial presentation, and 12 cases (17.1%) at relapse. Most frequently, codon R882 located in exon 23 was mutated, and 32 cases (91.4%) of these mutations were located within methyltrasferase domain.We evaluated the correlation of clinical and genetic characteristics with *DNMT3A* mutations. The frequency of higher white blood cell count (p= 0.044), M5 in FAB classification (p= 0.039), and intermediate risk karyotype (p<0.001) in patients with *DNMT3A* mutations were significantly higher than those in patients without them. Also, patients with *DNMT3A* mutations had a mutation in *NPM1* (p<0.001), *FLT3ITD* (p<0.001), and *IDH1/2* (p=0.016) more frequently.We next assessed the prognostic impact of *DNMT3A* mutations. For total cohort of patients with AML, complete remission rate and rates of relapse free survival (RFS) at 5 years were not statistically different between AML patients with and without *DNMT3A* mutations. However the overall survival (OS) at 5 years in patients with *DNMT3A* mutation (11.0%) was significantly lower than those in patients without them (28.9%) (p=0.003). Among the intermediate risk karyotype or *FLT3ITD* negative AML patients, RFS at 5 years were not statistically different between AML patients with and without *DNMT3A* mutations, but OS at 5 years in patients with *DNMT3A* mutation (intermediate risk karyotype: 12.6%, *Flt3ITD* negative: 11.6%) was significantly lower than those in patients without them (intermediate risk karyotype: 23.9%, p=0.035, *FLT3ITD* negative: 30.9%, p=0.009). Finally *DNMT3A* was an independent adverse prognostic factor by multivariate analyses (Hazard ratio 2.573, 95% CI 1.036-6.386, p=0.042). **Conclusions.** This study shows that *DNMT3A* mutation is an important adverse prognostic factor among intermediate risk karyotype or *FLT3ITD* negative AML patients. Recently *TET2* and *IDH* 1/2 gene mutations in *de novo* AML were reported. These genes including *DNMT3A* play an important role in epigenetic regulation of genes such as methylation etc, and mutations of these genes may be associated with leukemogenesis of AML. Now we are analyzing *TET2* mutations among same our AML cohort, and we will show the prognostic impact of *TET2*, *IDH1/2* and *DNMT3A* mutations in patients with *de novo* AML.

0067

EPIDEMIOLOGY AND OUTCOMES OF APL IN A LARGE POPULATION-BASED CANADIAN COHORT

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Background. Acute promyelocytic leukemia (APL) is a rare but highly curable sub-type of acute myeloid leukemia (AML). Recent epidemiologic studies suggest that APL incidence may be rising, and that outcomes of this disease are substantially worse than those reported in clinical trials. We hypothesized that in Canada: (1) that the incidence of APL is increasing and (2) in a universal health system with regional acute leukemia referral centres, outcomes would be as favorable as those suggested by clinical trials. **Aims.** To determine the incidence and mortality of APL using the population based Canadian Cancer Registry. **Methods.** New cancer diagnoses and deaths are required to be reported to provincial cancer registries in Canada. The Canadian Cancer Registry (CCR) collects data from provincial cancer registries, resulting in a national comprehensive, population-based database of cancer incidence and mortality. We reviewed all new diagnoses of APL in Canada between 1993 and 2007 to determine trends in incidence and mortality. **Results.** A total of 399 cases were identified, representing 3.01% of all AML cases. The age adjusted incidence of APL was 0.073 cases per 100,000. Incidence was higher in older patients (>50 years) (0.136/100,000, as compared to 0.057/100,000 for younger pts <50). There was an increased incidence in younger pts between 2005 and 2007 compared to the previous years (incident rate ratio 1.42, 95% confidence interval 1.07 - 1.88). For all patients, 30 day survival was 78.2%, and did not improve over the study period. In younger pts, 30 day survival was 89.3%, as compared to 64.5% in older adults. 5 year survival was 73.3% in younger adults, as compared to 29.1% in older adults. A logistic regression model was conducted in each subgroup, and there was no trend towards decreased early or late mortality with treatment in more recent years. **Conclusions.** In this, the largest complete national study of APL, the overall incidence was similar to previously published studies, but was higher in older adults. In younger adults, an increased incidence was noted in more recent years. The early death rate of 35.5% in patients over age 50 and 10.7% in patients younger than 50 are similar to previously published cohorts. This further confirms that early death rate (as a surrogate for therapy related mortality) is higher than suggested by clinical trials. Patient selection and rigorous attention to supportive care might account for this difference. The very high early death rate in older adults is concerning, and further efforts are required to determine how to improve outcomes for this group. We also recommend further close surveillance of APL in view of the recent increase in incident cases.

Acute myeloid leukemia - Clinical 2

0068

THE CLINICAL RELEVANCE OF BAALC AND ERG EXPRESSION LEVELS IN PEDIATRIC AML

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Background. Pediatric AML is curable in approximately 70% of patients. Its prognosis is determined by genetic aberrations and early treatment response, although 20% of pediatric patients lack cytogenetic aberrations (CN-AML). In adult CN-AML patients, prognosis is worse when there is high expression of either *BAALC* (Brain And Acute Leukemia Cytoplasmic; chromosome 8q22) or *ERG* (E26 transformation-specific-related; chromosome 21q22). **Aims.** We studied expression levels of *BAALC* and *ERG* in children in different cytogenetic subgroups aimed at improving risk group stratification and thereby also risk-adapted treatment and survival in this subgroup. **Methods.** Using gene expression profiling (Affymetrix Human Genome U133 Plus 2.0 Array) we studied *BAALC* and *ERG* expression in 294 *de novo* pediatric AML patients, who had been fully characterized for molecular aberrations. Validation was done using RT-qPCR, where we found high correlations between GEP and RT-qPCR for *BAALC* (R_s=0.809, P<0.001) and *ERG* (R_s=0.798, P<0.001) expression levels. *BAALC*^{high} was defined as above-median and *ERG*^{high} as above the 75th percentile. **Results.** The CBF-AML group had significantly higher *BAALC* expression levels (P<0.001) than non-CBF-AML. However, there was no difference in expression levels between the CN-AML group and all other cases. *ERG* expression levels were found to be higher in t(15;17) cases (P<0.001) than in all other cases. *ERG* expression was not associated with outcome in any subgroup, nor in the total cohort. In CN-AML, high *BAALC* expression was a poor prognostic factor for OS (*BAALC*^{high} vs *BAALC*^{low}; 47±11% vs. 76±10%; P=0.03), and a trend for EFS (29±10% vs. 50±11%; P=0.07) and CIR (62±11% vs. 37±10%; P=0.07). However, multivariate analysis, including *NUP98/NSD1* translocations and *NPM1* mutations, did not identify *BAALC* expression as an independent risk factor for OS in CN-AML.Summary/ **Conclusions.** Hence we conclude that *BAALC* and *ERG* expression have no prognostic significance in pediatric AML.

0069

PHASE I STUDY OF PLERIXAFOR IN COMBINATION WITH CLOFARABINE IN PREVIOUSLY UNTREATED OLDER ADULT PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA (AML) UNLIKELY TO BENEFIT FROM STANDARD INDUCTION CHEMOTHERAPY

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Background. Older patients with AML have inferior treatment outcomes including high treatment related mortality, lower CR rates and shorter remission durations. Clofarabine (CLO) is among the most active anti-leukemic agents in elderly AML patients with an approximate overall remission rate (ORR = CR + CRp) of 40%. AML cells express CXCR4 chemokine receptors which cause leukemia cell retention in the marrow microenvironment, where AML cells are protected from cytotoxic drugs. To achieve AML cell mobilization and sensitization to CLO, we designed a combination trial of plerixafor, a small-molecule CXCR4 antagonist, with CLO. Primary endpoint was to determine safety of this drug combination. **Methods.** This is a single arm Phase I study for adults with untreated AML who were ≥60 years with at least one of the following adverse prognostic factors: age ≥70 years, AHD, PS=2, and/or intermediate/unfavorable risk karyotype. Plerixafor was dose-escalated in a 3+3 design, starting at 240 mcg/kg, and proceeding to dose levels of 320 mcg/kg, and 400 mcg/kg. Plerixafor was given daily as subcutaneous (SQ) injection on days 1-5, 4-6 hours prior to CLO, which was given at doses of 30 mg/m^2 intravenously (IV) during induction and 20 mg/m^2 during re-induction/consolidation. Dose-limiting toxicities (DLT's) were assessed during the first cycle. Correlative laboratory studies included characterization of AML cell mobilization and immunophenotype changes. **Results.** The median duration of follow up in surviving patients at time of this analysis was 16 weeks. Median duration of remission (DOR) was 49

weeks. The median overall survival was 38 weeks. Of 13 enrolled patients, 6 (46%) achieved a response (4 CR, and 2 CRp), 5 had resistant disease (38%), and 2 were not evaluable due to early death from infectious complications (30 day all-cause mortality 15%). We did not notice any prolonged myelosuppression, the median time to ANC and platelet recovery was 29 and 25 days, respectively, without prolonged time to recovery at higher plerixafor doses. Sequential CBCs demonstrated mobilization of AML cells into the peripheral blood, accounting for a 107 ± 30% increase in peripheral blasts at 2 hours (n=8), a 218 ± 105% increase at 4 hours (n=9), and a 264 ± 94% increase at 8 hours (n=11) after plerixafor injection. Immunophenotype studies did not reveal any significant surface marker changes on the AML cells, particularly no CXCR4 up-regulation. Drug-related adverse events were nausea, febrile neutropenia, diarrhea, rash, and bilirubin and ALT elevations. Most treatment-related events were Grades 1-2 (except 2 cases of grade 3 ALT elevation). **Summary and Conclusions.** These data indicate that the combination of plerixafor with CLO is active and well-tolerated in treatment-naïve, older AML patients with ≥1 adverse prognostic factor. Safety data from this study are consistent with previously reported studies of single-agent CLO in older patients. Importantly, we did not notice any prolonged myelosuppression. Accrual into the expanded last dose cohort is expected to finish shortly, and the complete Phase 1 experience will be presented at the meeting. A Phase 2 study of plerixafor plus CLO is underway to determine the efficacy of this new drug combination (NCT01160354).

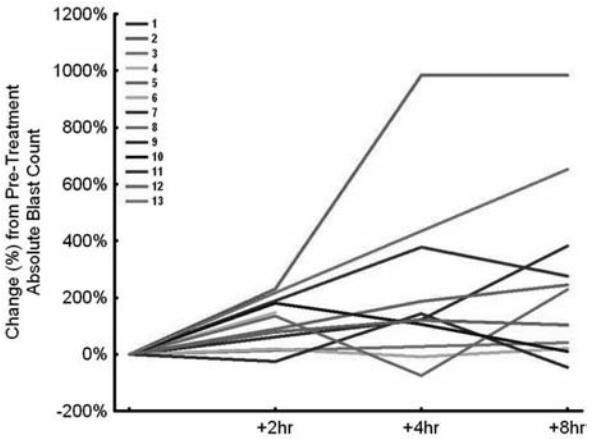

Figure 1. Blast increase (%) in the PB after plerixafor in individual AML patients.

0070

PREDICTION OF MORTALITY AND SURVIVAL OF PATIENTS WITH ACUTE MYELOGENOUS LEUKAEMIA IN THE INTENSIVE CARE UNIT - A SINGLE CENTER EXPERIENCE

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Background. Patients with haematological neoplasms including acute myelogenous leukaemia (AML) display a dismal outcome when transferred to Intensive Care Unit (ICU) for life-threatening complications. Therefore, it is crucial to identify parameters with prognostic significance. **Aims.** To identify potential risk factors for mortality and survival in patients with AML admitted to ICU. **Methods.** In critically ill patients with AML treated in the University Hospital of Muenster, Germany, between 11/2004 and 06/2011, the following demographic and clinical parameters were evaluated for risk prediction by an explorative approach: Age, sex, *de novo* versus secondary AML, cytogenetic and molecular risk according to the ELN 2010 classification, disease status (relapsed / refractory versus others), previous allogeneic stem cell transplantation (alloSCT), reason for ICU admission (sepsis versus others), paO2, mean arterial blood pressure, urine production, Glasgow coma scale (GCS), haematocrit, lactate and left ventricular ejection fraction (LVEF) at the time of ICU admission, as well as days between hospital admission and ICU admission and (for survival after ICU discharge only) days spent in ICU. Parameters were evaluated by univariate analysis for correlation with death during ICU and with survival after ICU discharge; only parameters with a *P*-value < .1 were selected for subsequent multivariate analysis. **Results.** Of n=187 AML patients admitted to our ICU, 108 (58%) succumbed while in ICU. Prognostic factors independently associated with death on ICU were: good risk according to ELN 2010 classification (Hazard Risk [HR]: 0.25; 95% CI: 0.09 - 0.67), hypoxia with paO2 < 72 mmHg (HR: 5.9; 2.6 - 12.5), sepsis (HR: 10.5; 4.7 - 23.8), lactate > 3 mmol/L (HR: 3.0; 1.4 - 6.3) and LVEF ≤ 60% (HR: 2.5; 1.18 - 5.3). Based on these parameters, a score predicting ICU mortality of AML patients was calculated, showing a goodness of fit of AUC=0.86 in the receiver operator characteristics (ROC), compared to 0.72 for the SAPSII score. Accuracy of prediction could also be demonstrated by comparing the median predicted ICU mortality with the observed mortality in quarters of patients by predicted mortality: 1st quarter, predicted 10% versus observed 18% (9/49 patients), 2nd quarter: predicted 49% versus observed 43% (19/44), 3rd quarter: predicted 77% versus observed 77% (37/48), 4th quarter: predicted 94% versus observed 94% (43/46). In comparison, the 79 AML patients (42%) surviving the ICU showed a 3-year survival of 64% (95% CI: 51 - 77%). Disease status, a previous alloSCT, days from hospital admission to ICU admission, days spent on ICU, an oliguria/anuria, GCS ≤ 7 and a hematocrit ≤ 25% were independent prognostic factors in a multivariate cox regression analysis. A prognostic index based on these factors was able to separate three distinct risk groups with 3-year survival rates of 100% in the low risk group (n=26), 67% (95% CI: 45 - 89%) in the intermediate risk group (n=26) and 24% (95% CI: 0 - 48%) in the high risk group (n=27), *P*<0.0001. **Conclusions.** With this large analyzed cohort of AML patients, we were able to identify independent risk factors for both mortality during and survival after ICU stay.

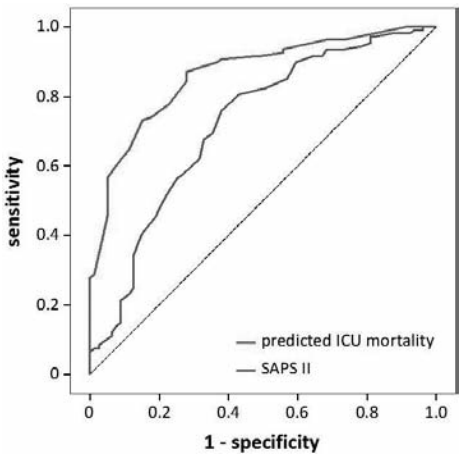

Figure 1. predicted ICU mortality.

0071

MICROTRANSPLANTATION AS POSTREMISSION THERAPY FOR ACUTE MYELOID LEUKEMIA: LONG-TERM FOLLOW-UP

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ABSTRACT**Background.** Despite best current therapy, about half patients with acute myeloid leukemia in first complete remission (AML-CR1) who had no HLA-identical donors relapsed. Whether the HLA-mismatched stem cell microtransplantation as a novel postremission therapy in such patients will improve survival and avoid graft-versus-host disease (GVHD) is still unknown. **Aims.** To evaluate effects and toxicity of the microtransplantation in AML-CR1 as a novel postremission therapy. **Methods.** 101 AML-CR1 patients (9-65 years old) from the four treatment centres, received programmed infusions of granulocyte colony-stimulating factor-mobilized HLA-mismatched donor peripheral blood stem cells following each of three cycles of high dose cytarabine chemotherapy conditioning without GVHD prophylaxis. Donor chimerism and microchimerism and $WT1^+$ $CD8^+$ T cells were analyzed. **Results.** The 6-year leukemia-free survival (LFS) and overall survival (OS) were 84.4%, and 89.5% in the low-risk group which was no significantly different from the intermediate-risk group (59.2% and65.2%, *p*=0.272 and p=0.308). The 6-year DFS and OS were 76.4% and 82.1% in the patients who received high dose of donor $CD3^+$ T cells (>$1.1x10^8$/kg) in each infusion which was significantly higher than those (49.5% and 55.3%, p=0.091 and p=0.041) in lower dose (<$1.1x10^8$/kg). No GVHD was observed in all of the patients. Sustained donor microchimerism (2

days to 1020 days) was detected in 20 of the 23 female patients who were available for detection of Y chromosome. A significant increase of $WT1^+$ $CD8^+$ T-cells (from 0.2% to 4.56%) was observed in 33 of the 39 patients with positive HLA-A*0201 antigen by a pentamer analysis. **Conclusions.** The microtransplantation as postremission therapy may improve outcomes but avoide GVHD in AML-CR1 patients.

0072

ORAL CLOFARABINE PLUS LOW-DOSE CYTARABINE IN PREVIOUSLY TREATED AML AND HIGH-RISK MDS PATIENTS =60 YEARS OF AGE

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Background. For many older patients with AML or high-risk MDS, the risks of standard induction chemotherapy may outweigh the benefits. The combination of intravenous (IV) clofarabine and cytarabine (ara-C) appears well tolerated and active in older or relapsed adults with AML. A randomized trial in untreated patients age ≥60 suggested that IV clofarabine plus low-dose ara-C (LDAC) was more effective than clofarabine alone. Oral clofarabine offers advantages to older patients and a phase II trial in higher-risk MDS patients confirmed the drug's activity, leading us to combine oral clofarabine with LDAC in a dose-escalation phase I/II study for patients age ≥60 with relapsed/primary refractory AML or high-risk MDS. **Aims.** Our goals were to estimate the maximum tolerated dose (MTD) and to assess complete remission (CR) rates. **Methods.** Oral clofarabine was administered daily on days 1-5 together with once daily subcutaneous (SQ) LDAC at 20 mgBID on days 1-10. Adults age ≥60 years with AML or high-risk MDS were eligible if they had primary refractory disease or failed one prior therapy and disease recurred within 1 year of CR date and had ECOG performance status 0-2. Informed consent was obtained from all patients. **Results.** Twenty-nine patients have been enrolled to date. Median age was 72 (range, 62-82) years. Eight patients (28%) had secondary AML. The median performance status was 1 (range, 0-2) and median time from diagnosis to trial entry was 12 (range, 2-67) days. Cytogenetics (SWOG criteria) were unfavorable in 13 patients (45%), intermediate in 9 (31%), favorable in 1 (3%), and unknown in 6 (21%). The MTD of oral clofarabine when given with LDAC was determined to be 20 mg and the dose-limiting toxicity was cardiac dysfunction. Number of cycles given: 23 patients - 1 cycle, 4 patients - 2 cycles, 1 patient - 3 cycles, 1 patient - 4 cycles. Twenty-six patients were treated as outpatients and 14 patients were hospitalized during the course of treatment including 11 for fever. Seven patients (24%) have achieved a CR. Based on previous MD Anderson data (Blood 1996; 88:756), the expected CR rate with HiDAC, FLAG *etc* would be 15%. Three patients (10%) achieved CR with incomplete blood count recovery and 1 (3%) a partial remission. Four of the 10 patients who achieved CR or CRi have relapsed at a median of 4 months. The other 6 have been in CR/CRi for a median of 3.5 months (range, 0-13.5 months). Fifteen patients (52%) died after treatment, 10 due toAML. **Conclusions.** Oral clofarabine at 20 mg daily for 5 days plus LDAC appears to be well tolerated and can generally be delivered in an outpatient setting. The CR rate appears higher than expected. While accrual is on-going, these results warrant further study.

0073

LATE PHYSIOLOGIC AND PSYCHOSOCIAL OUTCOMES AMONG LONG-TERM ADULT SURVIVORS OF ACUTE PROMYELOCYTIC LEUKEMIA

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Background. Since the introduction of all-trans retinoic acid (ATRA) and arsenic trioxide, acute promyelocytic leukemia (APL) has become the most curable adult leukemia with cure rates now exceeding 80%. In addition, APL affects younger patients compared to other subtypes of AML (median age at diagnosis 44 vs. 65). This has resulted in a growing population of APL survivors that did not exist just a few decades ago. However, little is known about the late effects of treatment on APL survivors. **Aims.** We conducted a single institution retrospective survey to examine the late physiologic and psychological outcomes among adult APL survivors. **Methods.** Patients with newly diagnosed APL treated at Memorial Sloan-Kettering Cancer Center between 1992 and 2007 were identified. Patients who are alive at 5 years or more from the date of diagnosis and remain in continuous remission were classified as APL survivors. Individual medical and pharmacy records were reviewed to analyze treatment-induced cardiac toxicity, second malignancies, and psychosocial outcomes as assessed by presence of depression, anxiety, social functioning and the use of anti-depressants and/or sedatives. **Results.** 52 adult APL survivors were identified with a median follow-up of 9 years (range, 5 - 19 years). The median age at diagnosis was 44 years. All received induction therapy with ATRA +/- anthracyclines. The median cumulative dose of doxorubicin isotoxic equivalents was 420 mg/m^2 (range, 0 - 792). Serial echocardiograms were obtained in 16 patients (30.8%) during the follow-up period. Twelve patients (23%) experienced late cardiac toxicity, consisting of congestive heart failure (n=2), left ventricular hypertrophy (n=1), and recurring episodes of syncope, dizziness, and palpitations (n=9). Two patients who experienced congestive heart failure had a known history of coronary artery disease and hypertension but with normal cardiac function prior to APL treatment. Chronic anxiety, depression and difficulty returning to work were documented in 13 patients (25%), 8 of whom required anti-depressants and/or counseling. Two patients developed second malignancies: localized prostate cancer 3 years after APL treatment at age 65, and metastatic lung adenocarcinoma 9 years after APL treatment at age 69. **Summary and Conclusions.** This study demonstrates an unusually high rate of long-term cardiac toxicity and chronic emotional and social difficulties among adult APL survivors. This result indicates that long-term follow-up should be standardized for adult leukemia survivors similar to those applied to childhood cancer survivors, which will allow studies to evaluate the actual incidence of adverse physiologic and psychosocial outcomes in a larger cohort. Because APL is the only highly curable subtype of adult AML with a relatively young median age at diagnosis, it is the best disease-model to focus on identification, prevention and control of the late and long-term adverse effects of leukemia treatments.

0074

FLUDARABINE-BASED INDUCTION THERAPY DOES NOT OVERCOME THE NEGATIVE PROGNOSTIC ROLE OF BAALC OVER-EXPRESSION IN ACUTE MYELOID LEUKEMIA

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Background. Despite progresses in understanding leukemia pathogenesis and in supportive care, the prognosis of acute myeloid leukemia (AML) remains dismal, as with conventional therapy long-term survival is attained in less than 30% of patients. Among factors negatively impacting on prognosis, multidrug resistance (MDR) protein ABCB1 (PGP) over-expression plays an important role. Inclusion of fludarabine in induction therapy overcome MDR effect, increasing remission rate and survival in ABCB1+ patients. However, less is known on the impact of fludarabine on other recently identified molecular alterations associated to negative prognosis. **Aims.** We evaluated the impact of various molecular factors on the outcome of 140 patients with AML treated at our Institution with a fludarabine-based induction protocol. **Methods**. One hundred forty consecutive patients with non-promyelocytic AML treated with a fludarabine-based induction chemotherapy and at least one consolidation course with high-dose cytarabine between 2004 and 2011, were included in our analysis. Blast cells immunophenotype and MDR proteins (ABCB1, ABCG2) expression were evaluated by multiparametric flow cytomety. Molecular analysis for FLT3-ITD and NPM1 mutations were performed by standard methods, and BAALC expression was calculated by real time PCR, with the cut off value set at 50th percentile. **Results.** Median age was 55 (range: 20-84) years, and 44 patients (31%) had secondary leukemia. Karyotype was evaluable in 130 patients: 7 (5%) favorable, 83 (64%) intermediate and 40 (31%) unfavorable. MDR proteins ABCB1 and ABCG2 were over-expressed in 39/140 (28%) and 60/140 (43%) cases, respectively. FLT3-ITD and NMP1 mutations were present in 38/140 (27%) and 36/140 (26%) patients, while BAALC was over-expressed in 61/140 (44%). After induction therapy, 101 (72%) patients achieved complete remission (CR), while 39 (28%) were refractory. In multivariate analysis, CR was negatively affected by secondary leukemia (P=0.003) and BAALC over-expression (P=0.035). Thirty-one of 101 (31%) patients relapsed, at a median of 14 months from diagnosis. Relapse probability was affected only by ABCG2 over-expression (P=0.03). Four-years overall survival (OS) was 49% (95% CI: 40-60). Lower survival probability was associated with older age (P=0.0001), high ABCG2 (P=0.003) and BAALC over-expression (P=0.02) while no association was found with FLT3 and NPM1 mutations or ABCB1 over-expression. However, subdividing patients according to FLT3, NPM1 and BAALC status, we could identify three groups with significantly different OS: FLT3-/NPM1+/BAALC± patients showed the best survival, FLT3+/NPM1-/BAALC±, FLT3+/NPM1+/BAALC+ or FLT3-/NPM-/BAALC+ patients had the worst survival, while FLT3-/NPM1-/BAALC- and FLT3+/NPM1+/BAALC- patients displayed intermediate OS (P=0.002). **Summary and Conclusions.** Our data suggest that BAALC

over-expression has a negative impact on CR attainment and OS in AML patients, irrespectively of cytogenetics and, to a lesser extent, FLT3 and NPM1 mutational status, whit the exception of the FLT3+/NPM1- subset. Fludarabine is confirmed to overcome the negative impact of high ABCB1 and FLT3-ITD mutation, but seems to have no impact on BAALC over-expression.

0075

CLINICAL AND BIOLOGICAL CHARACTERISTICS OF ISOCITRATE DEHYDROGENASE 1 AND 2 MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Background. Mutations of isocitrate dehydrogenase 1 and 2 (*IDH1 and 2)* are novel genetic alterations identified in the background of acute myeloid leukemia (AML). *IDH1 and 2* mutations act through the neomorphic production of 2-hydroxy-glutarate (2HG) resulting in the competitive inhibition of several 2-oxoglutarate (2OG) dependent enzymes, causing comparable methylation profile changes and gene expression patterns. On the other hand, some reports suggest that different *IDH* mutations may have distinctive clinical features and affect differently treatment outcome. **Aims.** To investigate the frequency, clinical associations and the prognostic effect of *IDH* mutations in the entire patient population, followed by the detailed analyses of particular *IDH* mutations in a Hungarian cohort of patients with AML. **Methods.** 376 AML patients (180 males/196 females; median age: 51; range: 16-93 years) diagnosed and followed between 2001-2009 were enrolled in the study. Remission and relapse rates, and survival were analyzed for 314 patients younger than 60 years and treated with curative intention. *IDH* mutations were screened using allele-specific PCR. Positive results for AS-PCR were confirmed by high resolution melting and sequencing. **Results.** *IDH1* and *IDH2* mutations were mutually exclusive, detected in 32 (8.5%) and 28 cases (7.5%). All mutations were in heterozygous form. In *IDH1*, R132C (n=14, 43.7%) and R132H (n=10, 31.2%) substitutions were the most frequent. In *IDH2*, R140Q (n=20, 71.4%) and R172K (n=8; 28.6%) were identified. *IDH* mutations combined were associated with: older age (p=0.001), higher average platelet counts (p=0.001), intermediate karyotype (p<0.0001), *NPM1* mutation (p=0.022) as well as with a lower mRNA expression level of *ABCG2* gene (p=0.006) at diagnosis. The same findings remained significant for the *IDH1* and *IDH2* mutants analyzed separately vs. *IDH1 and 2* wild type combined (n=316) comparisons, except for *ABCG2* expression in the *IDH1* comparison which showed only a tendency (p=0.062) and *NPM1* mutation in the *IDH2* comparison, which was not significant (p=0.17). Interestingly, particular *IDH* mutations differ in association with *NPM1* mutations: double *IDH* and *NPM1* mutations occurred in 14.3% (2/14) of *IDH1* R132C vs. 70% (7/10) R132H carriers (p=0.02) and in 47.4% (9/20) of *IDH2* R140Q vs. 0% (0/8) R172K carriers (p=0.02). Remission rate, relapse rate, overall survival (OS) were not different in *IDH1* mutant (n=24) vs. *IDH1 and 2* wild type (n=269) or in *IDH2* mutant (n=21) vs. *IDH1 and 2* wild type. *IDH1* R132H (n=9) negatively influenced OS compared to *IDH1 and 2* wild type (p=0.02) or to R132C (n=9, p=0.019). No differences were found comparing various IDH2 mutations with respect to treatment outcome. ***Summary and* Conclusions.** *IDH1 and 2* mutations are associated with distinct clinical characteristics, although alterations in NPM associations suggest differences between particular mutations. *IDH* substitutions are generally considered as a weak prognostic factors influencing survival in an inconsistent way in different AML subgroups, which may originate from different amino acid changes affecting outcome in opposite ways. Our observations may suggest that specific mutations might differ in the level of 2-HG production or they might act differently on other pathways besides blocking 2OG-dependent enzymes.

0076

ROLE OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MONOSOMAL KARYOTYPE

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Background. Monosomal karyotype (MK), defined as at least two autosomal monosomies or one single autosomal monosomy with one or more structural cytogenetic abnormalities, has been associated with dismal outcomes in acute myeloid leukemia (AML) in previous reports. **Aims.** We analyzed the clinicolaboratory characteristics and prognostic significance of MK in patients with AML and we also evaluated the possible role of allogeneic hematopoietic cell transplantation (HCT) in patients with MK. **Methods.** We retrospectively analyzed 749 patients with newly diagnosed AML, who received intensive induction chemotherapy between 1990 and 2010 at a single institute. Patients with acute promyelocytic leukemia were excluded in this study. Patients were classified as having good, intermediate, or poor risk cytogenetics according to the NCCN guideline, and MK status was also determined. **Results and Summary.** MK was found 71 patients (9.5%) and it was more frequent in 50 years or older patients (12.3%) or patients with secondary AML (19.1%) compared to patients younger than 50 years (7.1%; p=0.015) or patients with *de novo* AML (8.2%; p=0.001), respectively. MK was mostly associated with poor risk cytogenetics: 1/123 (0.8%) patients with good risk karyotype, 1/463 (0.2%) patients with intermediate risk karyotype, 9/59 (15.3%) patients with poor risk non-complex karyotype, and 60/104 (57.7%) patients with poor risk complex karyotype. Patients with MK had significantly lower initial leukocyte counts (p<0.001) and lower bone marrow blast percentage (p=0.039) than those without MK. Induction chemotherapy regimen was cytarabine plus daunorubicin or idarubicin in most patients. The rate for complete remission (CR) was significantly lower in MK (n=31, 43.7%) compared to non-MK (n=534, 78.8%; p<0.001). Allogeneic HCT in the first CR was performed in 14 patients with MK and 192 patients without MK. Overall survival (OS) and relapse-free survival (RFS) was significantly lower in MK patients compared to non-MK patients (OS, median, 6.5 vs. 25.7 months, p<0.001; RFS, median, 10.0 vs. 21.5 months, p=0.005). Multivariate analyses showed that MK was an independent risk factor for lower CR rate (hazard ratio [HR], 0.228 compared to intermediate risk cytogenetics, p<0.001), inferior OS (HR, 2.878, p<0.001), and inferior RFS (HR, 2.213, p=0.001). When only the patients with MK were analyzed, performance of allogeneic HCT in the first CR was independent prognostic factor for higher OS (HR, 0.374; p=0.032) and higher RFS (HR, 0.372; p=0.044). The median OS and RFS of the patients with MK who received allogeneic HCT in the first CR were 22.2 months and 16.8 months, respectively. **Conclusions.** Our study confirmed dismal outcomes of the AML patients with MK. Performance of allogeneic HCT in the first CR improved the outcomes of the patients with MK, but the survivals after HCT were still low.

Table 1. Multivariate analysis.

	OS		RFS	
Parameters	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, ≥60 years	2.038 (1.644-2.525)	**<0.001**	1.982 (1.513-2.595)	**<0.001**
WBC count, ≥60 (x10³/μL)	1.537 (1.221-1.935)	**<0.001**	1.501 (1.128-1.998)	**0.005**
Uric acid, ≥7mg/dL	1.431 (1.088-1.883)	**0.010**	1.617 (1.156-2.261)	**0.005**
Cytogenetics				
Good	0.777 (0.572-1.056)	0.107	1.076 (0.791-1.463)	0.641
Intermediate	1		1	
Poor				
Poor, MK-	1.826 (1.394-2.391)	**<0.001**	1.734 (1.238-2.429)	**0.001**
Poor, MK+	3.009 (2.244-4.035)	**<0.001**	2.171 (1.361-3.462)	**0.001**

0077

FERTILITY IN LONG-TERM SURVIVORS OF ACUTE MYELOID LEUKEMIA

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Background. Acute myeloid leukemia (AML) survival rates have improved considerably since the 1970:s, especially for younger patients. In Sweden today just over half of the patients diagnosed in their fertile age (20-44 years) are long-term survivors (surviving for more than five years). This creates a growing group of young adults living with potential sequelae of the disease or its treatment, and consequences on their family situation. The average age of giving birth to/fathering one's first child is also steadily rising, further increasing the group of young AML patients who have not yet had the children they want when they start their treatment. Risk factors for treatment-related infertility are well described mainly in leukemia of late childhood and adolescence. Few studies concerning the incidence of pregnancies in adult AML survivors have been published. **Aim.** To evaluate the impact of AML and its treatment on fertility and family situation in adult long-term survivors. **Methods.** Using the Swedish population registries, we identified 311 adult (≥20 years) patients who were diagnosed with AML within the Leukemia Group of Middle Sweden (LGMS) 1973-2003, and survived for more than five years. A questionnaire including items on reproductive concerns, family situation and infertility-related distress was used for data collection. One hundred of the 161 survivors (62%) still alive at study start in May 2010 and included in this study complet-

ed the questionnaire. After excluding women >45 years and/or postmenopausal women and men >55 years, 22 women and 38 men were included in the final analysis. Male patients were divided in two age groups: 20-45 years and 46-55 years. Written informed consent was obtained from all patients. **Results**. Despite a rather high (34 years) median age of women, 9 of them (41%) tried to get pregnant after treatment but only three succeeded. In addition, another of them adopted a child. Five (83%) of the unwillingly childless women reported "a moderate"or "a lot"of distress caused by this impairment. Among men in the same age group, all six who wanted children after treatment succeeded, three using assisted reproductive therapy and three without any additional assistance. None of the men 46-55 years old cryopreserved their sperm or tried to father a child. Among the patients that wanted children after AML treatment, 46% of women and 40% of younger men reported that they were not, or not fully, informed about fertility and fertility-related issues before or during treatment. In contrast, among men 46-55 years, none reported they would have wanted more information. **Summary and Conclusions.** Infertility after AML treatment caused a substantial degree of distress among females. Young men succeeded in fathering the children they wanted. Many young survivors felt that they were not adequately informed about therapy-related fertility aspects. Infertility among young female AML survivors remains an important clinical problem and there is a need for improved clinical counseling and education in this area.

0078

VECTORIZATION OF F14512, A NOVEL TARGETED CYTOTOXIC AGENT, TOWARDS LEUKEMIC CELLS: FUNCTIONAL BIOMARKER IMPLEMENTATION DURING A MULTICENTRIC PHASE 1 TRIAL IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background. F14512, a novel anticancer polyamine (spermine)-epipodophyllotoxin conjugate, is targeted towards tumor cells via the polyamine transport system (PTS). The PTS is an energy-dependent machinery generally hyperactive in cancer cells with a high demand for polyamines. In the absence of sufficient molecular knowledge on human PTS, a functional test was investigated to assess the uptake of F14512 into cancer cells. F17073, a proprietary fluorescently-labelled polyamine, was selected as a surrogate probe for PTS activity. Fluorescent microscopy and flow cytometry analysis demonstrated that F17073 uptake was modulated in competition with spermine or F14152, and thus specific of the PTS. As preclinical proof of concept, F17073 accumulation was correlated with the sensitivity to F14512. A flow cytometric-based assay was developed to measure F17073 uptake in acute myeloid leukemia (AML) fresh samples, for use as a functional biomarker in clinical trials. **Aims.** To assess the PTS activity in leukemic cells from patients accrued in the first multicentric phase 1 trial of F14512, as a functional biomarker of drug vectorization. **Methods.** After cross-validation of 6 hematology laboratories using a control blood pool, probe incorporation into patients' blast cells and lymphocytes was determined during the first phase 1 trial of F14512. Briefly, fresh peripheral blood (PB) and/or bone marrow (BM) samples collected at baseline were immediately transferred and processed in each hematology laboratory with a triplicate incubation (F17073 and CD45 Antibody ; 1hr ;37°C). Processed samples were shipped to a central hematology laboratory for flow cytometry reading within 24hr. The cellular accumulation of F17073 was measured as the mean fluorescence intensity detected in blast cells and lymphocytes. **Results.** During the cross-validation step, F17073-associated fluorescence was similar whether blood pool was processed in the central or in each hematology laboratory. The immediate processing of fresh samples associated with a centralised flow cytometry reading was therefore feasible in the multicentric phase 1 trial.As of december 2011, on 40 evaluable patients, F17073 fluorescence was higher in blast cells than lymphocytes in 80% of the samples, with no difference between the PB (n=35) or BM (n=32) origin of the samples. As compared to lymphocytes of the same patients, about 50% higher fluorescence was evidenced in blast cells ($p<0.001$) suggesting a preferential accumulation of the probe into AML cells.Potential relationships between PTS activity and F14512 cytotoxicity will be presented on mature data at the recommended dose. **Conclusions.** A functional test, usually highly dependent on cell viability and experimental conditions, has been here successfully implemented into a multicentric setting. Those results demonstrate that F17073 accumulates preferentially in leukemic cells for most of the AML patients eligible for F14512 administration. This functional biomarker confirms that F14512 is vectorized towards leukemic cells of such patients, and is therefore likely to further express its cytotoxic activity.

0079

RELEVANT PROGNOSTIC VALUE OF WILM'S TUMOR GENE EXPRESSION REDUCTION AFTER THE FIRST INDUCTION COURSE IN 82 CONSECUTIVE NON M3 AML PATIENTS

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Background. Most patients with acute myeloid leukaemia (AML) show increased levels of Wilms' tumor gene (WT1) expression at diagnosis. The aim of this study was to evaluate if the kinetics of reduction of WT1 expression may impact on relapse rate and disease free survival (DFS). **Patients and Methods.** In 82 consecutive non M3 AML patients (72 *de novo* and 10 secondary, median age 58 years, range 17-81 years) we studied WT1 gene expression on bone marrow samples at diagnosis and after one induction course. A quantitative Real Time PCR was used for the study of WT1 expression. Only patients with WT1 expression greater than 100 copies ($WT1/Ablx10^3$) at diagnosis were included. To evaluate the kinetics of reduction of WT1 expression we calculated the difference between the logarithm of WT1 value at diagnosis and WT1 level after induction (deltaWT1 = logWT1 at diagnosis - logWT1 post induction). DFS was evaluated from the date of the first CR to the date of the last follow-up for patient in CR or to the date of relapse or death for any cause. For patients submitted to bone marrow transplantation (BMT), the DFS was censored at the date of BMT. **Results.** DeltaWT1was less than 1 in 38 patients (46.3%), ranged between 1 and 2 in 22 (26.8%) and was ≥ 2 in 22 (26.8%). Hematologic response rate, relapse rate and DFS were significantly affected by deltaWT1. Among 38 pts with deltaWT1 < 1, 16 patients failed to achieve CR, 19 relapsed, 1 died in remission and 2 had a complete remission length of 1 and 3 months. Among 22 pts with a deltaWT1ranging between 1 and 2, 10 relapsed (DFS median 6 months, range 1-34 months), 5 patients were offered BMT in first CR and 7 maintain CR (median DFS 13 months, range 1-55 months). Among 22 patients with deltaWT1≥ 2 , 8 patients relapsed (median DFS 9.5 months, range 3-17 months), 2 patients underwent BMT and 12 are alive and disease free (median DFS 38.5 months, range 3-85 months). The projected DFS at 60 months of patients with deltaWT1 < 1, ≥ 1 and < 2, and ≥ 2 were 0 %, 27.8% and 51.9%, respectively ($p = 0.000$) (Figure 1).We tried to define a significant deltaWT1cut off level and found that deltaWT1 ≤ 1,5 was associated with higherfailure rate (response less than CR or relapse). Thirty-five of 38 (92%) patients with deltaWT1 < 1.5 did not respond or relapsed, whereas 19 of the remaining 44 pts (43,2%) with deltaWT1 > 1.5 ($p = 0.000$). **Conclusions.** our study shows that in AML patients response rate, relapse rate and DFS were affected by the kinetics of WT1 expression reduction. In particular patients with deltaWT1 < 1.5, though in CR, should be offered more intensive chemotherapy or BMT due to a high risk of relapse.

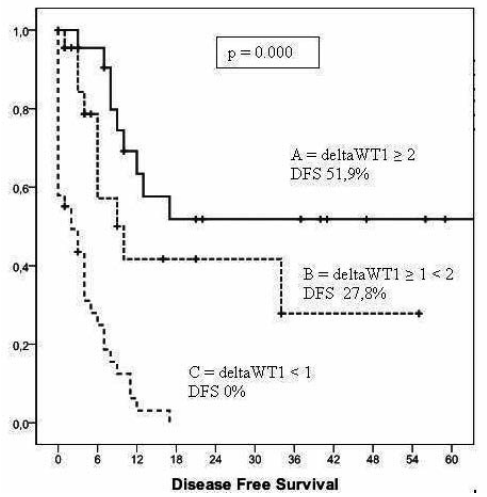

Figure 1. DFS according to delta WT1.

0080

SUBSEQUENT HSCT IN THE CLASSIC I STUDY ASSOCIATED WITH LONGER SURVIVAL IN PATIENTS WITH RELAPSED/REFRACTORY AML AFTER CLO+ARA-C OR ARA-C ALONE: A LANDMARK ANALYSIS

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Background. Outcome of elderly patients with relapsed/refractory acute myeloid leukemia (R/R AML) is extremely poor. Subsequent hematopoietic stem cell transplantation (HSCT) in patients with R/R AML may improve survival. **Aims.** We have previously reported the efficacy and safety outcomes of the CLASSIC I study in older patients with R/R AML (Kantarjian, EHA 2011). In this report, we evaluate the outcomes of subsequent HSCT after Clo+Ara-C or Ara-C alone in the CLASSIC I trial. **Patients and Methods.** CLASSIC I was a prospective, randomized, double-blind, placebo-controlled trial comparing Clo+Ara-C to Ara-C alone in patients ≥55 years old with R/R AML. Patients were randomized to either Clo 40 mg/m^2 or placebo followed by Ara-C 1 g/m^2 for 5 consecutive days. While the primary endpoint of overall survival in the CLASSIC I study did not differ between treatment arms, there were statistically significant improvements in the Clo+Ara-C arm in secondary efficacy endpoints (ORR, CR,EFS)In this post-hoc analysis, patient characteristics and safety were analyzed for those patients who underwent HSCT. We performed a series of landmark analyses to assess the extent of association (rather than cause and effect) between HSCT and overall survival (OS).

Table 1. Impact of HSCT on OS and HSCT-Related Safety.

	Overall (N=66)	CLO+Ara-C (N=34)	Ara-C (N=32)
Impact of HSCT on Overall Survival; HR (95% CI)			
Naïve estimate	0.30 (0.22, 0.40)	--	--
Landmark analysis across groups on FAS patients at 4 months	0.59 (0.37, 0.92)	--	--
Landmark analysis by group on CR patients at 4 months	0.72 (0.41, 1.27)	0.81 (0.40, 1.63)	0.61 (0.23, 1.60)
Landmark analysis by group on CR patients at 8 months	0.49 (0.31, 0.77)	0.50 (0.24, 1.01)	0.40 (0.16, 0.99)
HSCT-Related Safety; n (%)	**N=66**	**N=34**	**N=32**
100-day post-HSCT mortality	9 (13.6%)	4 (11.8%)	5 (15.6%)
Acute GVHD	11 (16.7%)	5 (14.7%)	6 (18.8%)
Acute Grade ≥3 GVHD	3 (4.5%)	1 (2.9%)	2 (6.3%)
Chronic GVHD	1 (1.5%)	1 (2.9%)	0 (0.0%)
VOD Complications	1 (1.5%)	0 (0.0%)	1 (3.1%)

HSCT, hematopoietic stem cell transplantation; FAS, full analysis set; CR, complete remission; GVHD, graft-vs-host disease; VOD, veno-occlusive disease

Results. Among 320 patients with centrally confirmed AML(age, median 67 yrs), 66 (21%; median age 63 years) underwent subsequent HSCT [Clo+Ara-C arm: n=34; Ara-C arm: n=32]. Of which, 47 patients (71%) proceeded to HSCT in CR; 36 in CR from study drug [Clo+Ara-C arm: n=22 and Ara-C arm: n=14 patients] and 11 in CR from additional therapy. 64 patients received an allogeneic transplant [sibling donor: n=46 (70%), unrelated donor: n=18 (27%)] and 2 (3%) were autologous transplants. Preparative regimens included myeloablative in 23 (33%) patients and reduced intensity conditioning/non-myeloablative regimens in 43 (66%) patients. Median time to HSCT was 3.8 months (range 1.6-14.8 months) from the initiation of the study drug, Multiple analyses were consistent with a survival benefit of HSCT - a naïve estimate comparing OS in patients with and without HSCT [HR 0.30 (95% CI 0.20, 0.40) *p<.0001*]; a 4-month landmark analysis comparing OS past 4 months in patients with and without HSCT [HR 0.59 (95% CI 0.37, 0.92) *p=.023*]; and landmark analyses assessing the impact of HSCT on OS for patients who achieved CR at 4 months [HR .72 (95% CI .41, 1.27) *p=0.255*] and at 8-months [HR 0.44 (95% CI 0.25,0.76) *p=.004*]. Although there was evidence that HSCT was associated with longer survival, there was little evidence of a differential effect across the treatment arms (interaction p=0.32). Post-HSCT safety findings were similar across treatment groups (Table 1) and raised no new concerns. **Conclusions.** Approximately 20% patients (median age 63 yrs) with R/RAML underwent subsequent HSCT in the CLASSIC I trial. More patients in the Clo+Ara-C arm proceeded to HSCT in CR from study drug. Overall, this post-hoc analysis of the CLASSIC I study demonstrated that subsequent HSCT in elderly patients with R/RAML was feasible and was associated with longer survival; though there was little evidence of a differential effect across treatment arms.

0081

BAALC OVEREXPRESSION IN AML IS ASSOCIATED WITH POOR PROGNOSIS IN ALL CYTOGENETIC RISK CATEGORIES

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Background. In the past years many molecular alteration have been associated with prognosis in adult myeloid leukemia with normal cytogenetic (CN-AML). Among them, overexpression of brain and acute leukemia cytoplasmic (BAALC) gene confers poor prognosis. Less defined is its role in AML with cytogenetic abnormalities. **Aims.** Objective of this study was to evaluate the role of BAALC over-expression in the outcome of adult patients with AML, irrespectively of karyotype. **Methods.** We analyzed 210 patients with AML treated at the Division of Hematology of Udine between 2005 and 2011. Median age was 60 years (range: 20-87) with 102 males and 108 females. One hundred forty patients had *de novo* AML while in 73 cases leukemia developed from a previous myeloproliferative disease or after exposure to chemo/radiotherapy. Karyotype at diagnosis was available in 186 patients: favorable in 23, intermediate in 109 (normal in 79) and unfavorable in 54. Quantitative BAALC expression was determined by real-time PCR and expressed in arbitrary units after normalization to ABL; the cut off value between high and low expression was set at 50th percentile. **Results.** BAALC was overexpressed in 89/210 (42%) patients. No association was found between BAALC positivity and sex, age, type of leukemia (*de novo* vs secondary), WBC count, FLT3-ITD status or CD56 expression. High BAALC expression was associated with CD34 positivity (71/89, 80%), while among 121 BAALC-negative patients only 49 (40%) were CD34+ (P<0.00001). Considering cytogenetic subgroups, BAALC overexpression was lower in patients with favorable karyotype (4/23, 13%) than in all others cytogenetic groups (67/163, P=0.05). Considering intensity of expression, higher BAALC levels were found in CD34+ vs CD34- cases (44.79±89.6 vs 9.05±31.6, P=0.0004) and in patients with intermediate karyotype, compared to those with favorable or unfavorable abnormalities (35.8±85.6, vs 10.2±25.6, P=0.04 and vs 14.3±29.4, P=0.05). One hundred ninety-one out of 202 patients (8 cases with promyelocytic leukemia were excluded from analysis) were evaluable for therapy response. In univariate analysis, BAALC overexpression showed a negative impact on complete remission achievement (P=0.002), as well as age, CD34 expression, and secondary AML; CD34+, age and BAALC retained their statistical significance also in multivariate analysis. BAALC did not influence relapse probability, nonetheless had a strong impact on overall survival (OS): 3-years OS was 50% (95% CI: 38-62) in BAALC negative patients, compared to 36% (95% CI: 24-47) in patients over-expressing BAALC (P=0.001). Karytotype per se did not influence survival probability, but subgrouping patients according to cytogenetic risk and BAALC expression we could identified three groups with different prognosis: favorable karyotype + low BAALC (3-years OS 75%, 95% CI: 64-87), intermediate karyotype irrespective of BAALC and unfavorable karyotype + low BAALC (3-years OS 48%, 95% CI: 38-56) and patients with favorable or unfavorable cytogenetic + high BAALC (3-years OS 34%,, 95% CI: 27-43) (P=0.02). **Summary and Conclusions**. BAALC over-expression identified AML patients with poor prognosis in all the cytogenetics group. Though relatively rare, positivity of BAALC in patients with favorable or unfavorable karyotype significantly worsened survival.

0082

A PHASE I TRIAL OF LENALIDOMIDE IN COMBINATION WITH INTERMEDIATE DOSE CYTARABINE (IDC) IN RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background. Lenalidomide, an oral immunomodulator, has been studied as a single agent in the treatment of relapsed/refractory AML. In a recent study, five (16%) of 31 patients (pts) achieved complete remission (CR), and all three pts with karyotype aberrations achieved cytogenetic CR. The drug was safely escalated to 50 mg daily for 21 days and was active with relatively minimal toxicity (Blum et al, JCO 2010). These low single-agent CR rates suggest that combining lenalidomide with chemotherapy might improve outcomes. Since IDC induces CR in approximately 17% of relapsed/refractory AML pts (Faderl et al, JCO 2012), we performed a phase I study to assess the safety and preliminary efficacy of IDC followed by lenalidomide in this patient population. **Aims.** To determine the maximum tolerated dose (MTD) of lenalidomide following IDC therapy in relapsed/refractory AML patients in the dose finding phase. To evaluate the safety and preliminary anti-leukemic activity of this combination in the dose expansion phase. **Methods.** The study was designed as a two phase study: a dose finding, and expansion phase. IDC was administered at 1.5 gm/m^2/day on days 1-5 of a 28 day cycle followed by lenalidomide initially given days 6-10 (6-10d) and then days 6-26 (6-26d). Lenalidomide dose range was 25mg, 15mg and 10mg. A maximum of two such inductions were allowed. Once CR was achieved, lenalidomide maintenance was then given daily for 28 days until disease progression. **Results.** As of January 20th 2012, 20 pts with a median age of 70 (range 34-79) years have been accrued to the dose finding arm of this study. Two patients have insufficient follow-up to report treatment results and adverse events. Thirteen pts (72%) had received more than two previous therapies. The number of pts with dose limiting toxicities (DLTs) at each dose level (DL) was: 0/4 pts in DL 1 (25 mg/d; 6-10d), 4/6 all grade 3 non-blistering macular-papular rash in DL 2 (25 mg/d; 6-26d), 3/7 pts grade 3 AST, grade 3 anorexia, grade 3 bilirubin in DL 3 (15 mg/d; 6-26d), and 0/3 pts in DL 4 with no DLT at present time and 2 pts with no information available (10 mg/d; 6-26d). Severe (grade 3-5) adverse events (SAEs) were observed in 17 of 18 pts. The most frequent hematological SAEs were thrombocytopenia (89%). anemia (83%) and neutropenia (61%). The most frequent non-hematological SAEs included nausea and vomiting (61%), skin rash (39%) and fatigue (33%). Two patients achieved CR and 1 patient demonstrated CR without platelet recovery (CRp) for an overall CR/CRp rate of 17% (3/18 evaluable patients). Three patients had stable disease. **Conclusions.** IDC and lenalidomide can safely be administered to refractory/relapsed AML pts. The dose expansion phase is ongoing at 10mg daily and will be reported at the meeting.

0083

ISTH DIC SCORE = 6: A NEW PREDICTOR OF EARLY DEATH IN ACUTE PROMYELOCYTIC LEUKAEMIA

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Background. Despite the improvements in acute promyelocytic leukemia (APL) treatment, early death (ED) remains a problem, with recently reported rates between 17 and 29%. **Aims.** To report ED in 49 *de novo* APL patients and to identify pretreatment factors predictive for ED. **Methods:** We retrospectively analyzed data on ED in 49 newly diagnosed, t(15;17)(q22;q12) or PML-RARA positive APL patients (median age 42 years, range 19-69; 23/26 female/male ratio) managed in the Clinic of Hematology from 2004 to 2011 with all-trans retinoic acid combined with anthracyclines. Patients with International Society of Thrombosis and Haemostasis Scoring System for diseminated intravascular coagulation (ISTH DIC score) ≥5 were considered to have overt DIC. ED was defined as death from any cause from the first day of hospitalization up to 30 days from therapy initiation. **Results.** Median time from the first symptoms to diagnosis was 20 days (range: 5-90). Pretreatment patients characteristic were as follows: ECOG PS 1, 2, 3, 4 had 17/49 (34%), 16/49 (33%), 14/49 (29%) and 2/49 (4%) patients respectively; median WBC count 3.9x10^9/L (range: 0.6-183); median platelet count 29x10^9/L (range: 4-101); hypergranular form in 43/49 (88%) patients; Sanz' s risk stratification: high 19/49 (39%), intermediate 19/49 (39%), low 11/49 (22%); median D-dimer 2426 µg/L (range 551-11340); median PT 62% (range 34-129); median fibrinogen level 2.35 g/L (range 0.57-6.5). DIC was confirmed in 42/49 (86%) patients, median ISTH DIC score was 6 (range: 2-7). Bleeders at diagnosis had higher WBC (29.3x10^9/L vs. 9.4x10^9/L, P=0.02), higer percentage of peripheral blood promyelocytes (36% vs. 14%, P=0.04) and higer median ISTH DIC score (6 vs. 4, P=0.0007) comparing to the non-bleeders. ISTH DIC score ≥5 was predictive for bleeding (P=0.034208).Therapy **Results.** ED occured in 10/49 (20.4%) patients due to: differentiation syndrome (DS) 3 (30%), endocranial bleeding 5 (50%) and infection 2 (20%). We did not register any death before diagnosis or due to misdiagnosis, but 3/49 (6%) of patients died before therapy initiation. Predictors of ED were: WBC >10x10^9/L (P=0.05), ECOG ≥3 (P=0.0006), fibrinogen level <2 g/L (P=0.02617), PT <50% (P=0.007292), ISTH DIC score ≥6 (P=0.00529) and DS occurrence (P=0.04). It is of note that DS occurred in 12 (27%) of patients, severe form in 9/12 (75%). **Conclusions.** High ED rate in our series is in line with current reports. Our results suggests that ISTH DIC score is a more sensitive predictor for ED compared to single parameters such as fibrinogen level, PT and D-dimer and can be used together with Sanz' s score to identify patients at very high risk for ED. Moreover an integrative prognostic risk score including WBC, ISTH score and ECOG PS might be even more sensitive. Further large population based studies for testing new score systems and refinements therapeutic approaches for APL are needed.

0084

PHARMACODYNAMIC ASSAYS DEMONSTRATE NAE PATHWAY INHIBITION FOLLOWING ADMINISTRATION OF MLN4924 IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. NEDD8 activating enzyme (NAE) initiates the NEDD8 conjugation pathway and is required for activity of cullin ring ubiquitin ligases (CRLs). Activation of CRLs controls timed degradation of substrates involved in cell cycle regulation, signal transduction, DNA replication and stress responses. MLN4924 is an investigational, small molecule NAE inhibitor which forms a covalent adduct with NEDD8, thereby blocking ubiquitination and proteasomal degradation of CRL substrates such as chromatin licensing and DNA replication factor 1 (CDT1). Preclinical tumor models treated with MLN4924 resulted in tumor regression and supported Phase I clinical development. **Aims.** Evidence of target inhibition and downstream pathway modulation was a secondary objective in the MLN4924 AML phase I study C15003. Patients were treated on days 1, 3, 5 of a 21 day cycle at doses ranging from 25 to 83 mg/m^2. Required bone marrow aspirate or biopsies (BMA) were taken at screening and on day five at 3-6 hours post dose. Here we describe the development and clinical implementation of IHC pharmacodynamic (PD) assays measuring MLN4924-NEDD8 covalent adduct (adduct) and CDT1 expression in pre and post dose BMAs from AML patients. **Methods.** THP-1 AML tumor xenograft models were treated with MLN4924. Tumors were collected for IHC at multiple post-dose time points. CDT1 and adduct antibodies were detected with a HRP polymer or a biotin labeled secondary, respectively. Antibody staining was quantified as a percent positive pixel count. The CDT1 assay for patient BMA biopsies was supplemented with a cocktail of CD34 and CD117 antibodies for tumor cell identification. Six slides for each biopsy were stained and Definiens software algorithms used to calculate the percent CD34/CD117 cells positive for CDT1. A mixed effects model was used to estimate the within subject coefficient of variation (CV) in tumor CDT1 levels. A population PK model was used to predict cumulative MLN4924 plasma AUC at day 5. **Results.** Pre-clinical AML models treated with MLN4924 demonstrated presence of adduct within 30 minutes lasting up to 24 hours post dose, indicating that MLN4924 rapidly distributes and persists in tumor tissue. IHC assays detected accumulation of CDT1 in xenograft tumors at 2-8 hours post treatment, demonstrating successful inhibition of NAE. IHC staining for adduct in BMA pairs from 21 patients confirmed presence of drug in 100% of the post-dose BMAs and absence pre-dose. Statistical assessment of the CDT1/CD34/CD117 IHC assay identified the minimum evaluable tumor burden with 20 out of 21 BMAs containing sufficient tumor. Sixteen of the twenty patients (80%) showed a statistically significant ($p<0.05$) increase in CDT1 expression in the post dose sample with a fold change of 1.48 or greater including 3/3 patients who achieved a complete response in the PD evaluable cohort. PK data was available for 18/20 patients however; no statistical correlation between CDT1 fold change and MLN4924 plasma AUC was identified. **Summary.** These data demonstrate inhibition of NAE and downstream pathway modulation in BMA from patients with AML who were treated with MLN4924 on days 1, 3, 5 in a phase I clinical trial at doses as low as 25 mg/m^2.

0085

CXCR4 MAY BE A PROGNOSTIC MARKER IN ACUTE MYELOGENOUS LEUKEMIA (AML)

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Background. CXCR4 and its ligand CXCL12 are involved in the maintenance of homeostasis of the homing of hematopoietic stem-cells in the bone-marrow microenvironment. In leukemia, CXCR4/CXCL12 axis is known to play a role in the attraction of leukemic blast cells in the bone marrow niches creating an occult source of minimal residual disease that could be responsible for leukemia relapse over time. **Aims.** This study analyses the CXCR4 expression on leukemic blast cells and its prognostic role evaluating disease-free (DFS) and overall survival (OS) of the patients with AML. Methods: Bone marrow samples at diagnosis from 84 consecutive patients with AML were analyzed by flow-cytometry (FACS) for the expression of CXCR4 on leukemic blasts. The cutoff of 10% was used in order to distinguish positive (CXCR4 >10%, Group A) from negative (CXCR4 ≤10%, Group B) cases to correlate antigen expression and survival outcome of the patients. DFS and OS were estimated by the Kaplan-Meier method, while survival curves were compared by the log-rank test. **Results.** The median age was 67 years (range, 17-86). Fifty-six patients (67%) suffered from a de novo AML, whereas 28 (33%) showed a previous hematological disorder (sAML from myelodysplasia, n=22; therapy related-AML, n=3; sAML from myeloproliferative disease, n=3). According to cytogenetics and molecular results, prognosis was favorable in 13, intermediate in 22 and unfavorable in 27 patients. 22 patients were unclassifiable. In most cases treatment consisted of cytarabine+anthracycline based induction regimen (group A, n=16; group B, n=41), while 19 (23%) patients received other cytoreductive therapies (hydroxyurea, n=8; low-doses cytarabine, n=4; 5'-azacitidine, n=7). Three patients received the best supportive care. Thirty-eight (45%) patients achieved the complete remission. Twenty patients were eligible for a stem cell transplant (SCT): 11 patients received allogeneic SCT, while 9 received autologous SCT. Median OS of the whole patient population was 12 months, while at the last f-up on December 2011 the median DFS was not reached. Group A (n=29) and Group B (n= 55) showed a median expression of CXCR4 of 24% (range, 11%-92%) and 3%, respectively. Median OS in the Groups A and B were 6 and 21 months, while DFS was 21 months for Group A and was not reached for Group B. By the log-rank test a significant correlation was found between the two Groups both for OS (p<0.011) and DFS (p<0.031). OS and DFS Hazard ratio were 2.298 (CI: 1.216-4.345) and 2.991 (CI:0.9185-9.736), respectively. **Conclusions.** Our findings showed a striking correlation between CXCR4 expression and the overall survival time in a heterogenous patient population affected by AML. These results agree with the reported biological role of the CXCR4/CXCL12 axis concerning the protection of leukemic cells in the hematopoietic niches. If these data will be confirmed in a larger cohort of patients, CXCR4 detection could be considered useful during the diagnostic work-up in AML.

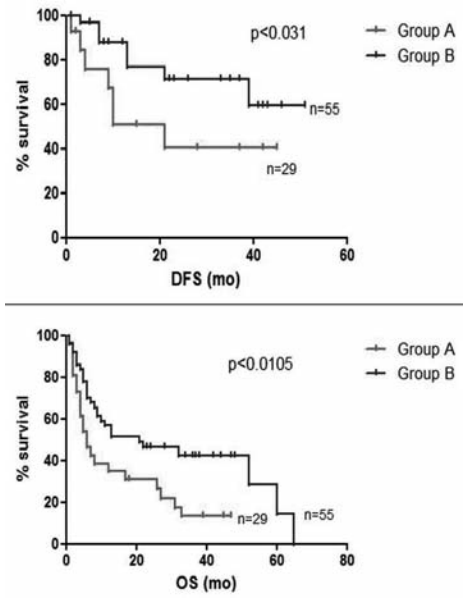

Figure 1.

0086

ALLOGENIC STEM CELL TRANSPLANT (ASCT) AS INITIAL SALVAGE FOR PATIENTS (PTS) WITH ACUTE MYELOID LEUKEMIA (AML) REFRACTORY TO HIGH-DOSE CYTARABINE-BASED INDUCTION CHEMOTHERAPY

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Background. Outcomes of pts with AML who are refractory to High-dose Cytarabine (HiDAC) based induction are dismal. ASCT as initial salvage may be effective and potentially superior to repeat induction with combination chemotherapies in such pts. **Methods.** 1597 AML pts were treated with HiDAC-based induction at our institution between 1995 and 2009 and 285 primary refractory pts were identified. 28 (10%) of these underwent ASCT as initial salvage and were reviewed. **Results.** Median age was 56 years (36 to 77) and 50% were males. Median ECOG PS was 1 (0-2). Antecedent hematological disorders were present in 14 pts (50%). Median white cell count, hemoglobin, platelet count and bone marrow (BM) blast percentage at diagnosis were 3.5 x 10^9/L (0.6 to 73.6), 7.8 g/L (6.5 to 11.4), 44x10^9/L (9 to 615), and 43% (6-82), respectively. 9 pts (32%) had complex cytogenetics. FLT3 mutations were identified in 2 (15%) of 13 evaluated pts. All pts were refractory to HiDAC-based induction. HiDAC was combined with an anthracycline in 16 pts (57%) and non-anthracycline in 12 pts (43%).Median time from induction to ASCT was 76 days (28 to 184). Median BM blast and peripheral blast at ASCT were 28% (3 to 82) and 4% (0 to 41). 21 pts (75%) had matched related donors (18 sibling and 3 haploidentical) and 7 (25%) had matched unrelated donors. Conditioning regimens were melphalan-based, busulfan-based, fludarabine-based or others in 7 (25%), 10 (36%), 8 (29%) and 3 pts (10%); respectively. Complete remission (CR) was achieved in 23 of 28 pts undergoing ASCT (CR = 82%) with median time to CR of 31 days (26 to 134). 12 pts relapsed with median time to relapse of 5 months (2 to 19). 8 pts remain alive with median follow up of 80 months (28 to 118). Median overall survival (OS) for the entire group is 20 months (5 to 118). In historical series of pts with AML refractory to HiDAC-based induction, salvage chemotherapies induced CR rates of 22% with median OS of 4 months. **Conclusions.** Initial salvage with allogenic SCT is feasible and yields superior outcomes to salvage chemotherapy in primary HiDAC refractory AML pts. Randomized studies are warranted to further explore this treatment option.

0087

G8 OR MULTIMODAL GERIATRIC ASSESSMENT (MGA) FOR THE SCREENING OF OLDER PATIENTS WITH MALIGNANT HEMOPATHIES ?

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Background. Although malignant hemopathies among older patients remain curable diseases, in some cases, their treatment can precipitate geriatric syndromes. To manage this risk, hematologists have to detect unsuspected health problems, to estimate survival and to predict tolerance to the treatment in order to optimizer the treatment (full dose chemotherapy, reduced-dose or palliative treatment). Growing evidences suggest that a Multimodal Geriatric Assessment (MGA) could help hematologists to achieve this goal but is time consuming and expensive. Short-screening tool (G8) could allow screening for fragile patients but is under evaluation. **Aims.** 1) To evaluate the respective usefulness of G8 and MGA to predict mortality in a selected population of consecutive « fit » older inpatients referred for the treatment of malignant hemopathies in a cancer centre. 2) To assess the usefulness of G8 to predict the initial treatment choice and the tolerance to chemotherapy. **Methods.** Between October 2009 and February 2012, a G8 and a full MGA assessment were performed in 60 consecutive older inpatients (≥65 years). These patients were considered «fit » enough by the referral physicians to receive chemotherapy. We excluded from the analyses patients for whom the disease does not require treatment or patients who refused the treatment. The normal cut-off for G8 assessment has been defined ≥15/17. Patients' initial treatment choice, tolerance to chemotherapy (therapeutic changes during treatment), death and cause of death were extracted from medical files. **Results.** G8 screening score was abnormal in 85% (n=51) of our inpatients. Differences between normal and abnormal G8 scores are not associated with one year survival (p=0.665). Causes of G8 abnormal score were primarily denutrition and psychological distress. MGA showed that a probable mild cognitive disorder (MMSE) (p=0.013) and a slow motion (Time Up and Go test) (p=0.014) are associated with a higher risk of death in the first year of treatment. Difference between normal and abnor-

mal G8 scores was curiously not associated with initial treatment choice (p=0.757) and tolerance to chemotherapy (p=0.481). **Conclusions.** Among older inpatients with hematological malignancies, G8 may be too sensitive to select older patients susceptible to benefit from full dose chemotherapy. In our small series of «fit» older patients, a poor G8 score does not allow to predict tolerance to chemotherapy and not translate into a worse survival. The leading cause of death (92%) remains the progression of the disease. Prospective trials are needed to determine whenever a lower threshold of G8 score could better identified -in malignant hemopathies- patients at risk of treatment related death. Prospective trials are also needed to assess the usefulness of MGA and to improve multidisciplinary care of older patients with malignant hemopathies.

0088

BURDEN OF ILLNESS OF FLT3-MUTATED ACUTE MYELOID LEUKEMIA (AML) IN THE UNITED STATES

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Objective. Patients with FLT3-mutated AML have poor prognoses due to short survival, high incidence of relapse, and a lack of durable effective treatment options. Limited information is published on the burden of AML, especially for FLT3-mutated disease. **Aims.** This study reviewed the literature and estimated the burden of FLT3-mutated AML in the US. **Methods.** A systematic literature review was conducted in PubMed to identify relevant publications from 2000-2011. 607 citations were identified and 35 articles abstracted. Epidemiologic data were also sought from the Surveillance, Epidemiology and End Results (SEER) database. Estimates of the epidemiology of FLT3-mutated AML were calculated from SEER data and US Census projections. An Excel model was developed to estimate the BOI of FLT3-mutated AML. Resource utilization estimates were obtained from the literature and expert opinion. **Results.** No direct estimates for the incidence or prevalence of FLT3-mutated AML in the US were identified in the literature. The prevalence of AML in 2008 was obtained from SEER data (27,813 patients aged ≥20 years). In 2010, it was estimated that 784 adults <60 years and 1,622 adults ≥60 years were diagnosed with FLT3-mutated AML.In patients age <60 years, the literature reported FLT3 mutations in up to 30% of AML cases. Per the literature review, FLT3-mutated AML is associated with a poorer prognosis: median overall survival estimates of 15.2-15.5 months for patients age <60 years, compared to 19.3-28.6 months for patients with FLT3-WT disease. Five-year survival for age <60 years ranged from 15% for high FLT3/ITD levels to 31% for low FLT3/ITD levels vs. 42% for FLT3-WT.Three studies were identified which reported information on the impact of AML on quality of life (QoL), though none described the QoL impact of FLT-3 mutated AML. One study specifically examined the <60 year old population, noting that patients receiving stem cell transplants had significantly worse long-term impact on QoL vs. patients receiving conventional chemotherapy. No studies quantifying the impact of AML on productivity were identified.The US BOI of FLT3-mutated AML was estimated at $251 million in 2010, including $191 million in direct costs and $60 million in lost productivity. Inpatient hospitalizations accounted for 36% of direct costs and stem cell transplants for 38%. The cost per newly diagnosed patient with FLT3-mutated AML <60 years was estimated at $114,193 vs. $105,819 for patients with FLT3-WT AML (statistical significance not tested). For newly diagnosed patients <60 years, the burden of FLT3-mutated AML was estimated at $90 million in 2010. A sensitivity analysis using American Cancer Society incidence data (n=13,780) yielded an estimate of $125 million in 2010 for newly diagnosed patients <60 years with FLT3-mutated AML. As early mortality and early retirement were not included in indirect costs, these costs are likely underestimated. **Conclusions.** FLT3-mutated AML potentially represents a greater per-patient burden than FLT3-WT AML due to shorter survival and greater use of stem cell transplantation. Investigational treatments targeting the FLT3 mutation may provide additional therapeutic options, with the potential to improve clinical outcomes.

Cellular Immunotherapy and vaccination

0089

EFFECTIVE PROPHYLAXIS OF CMV DISEASE IN RECIPIENTS OF ALLOGENEIC HAEMOPOIETIC STEM CELL TRANSPLANTS USING ADOPTIVE T CELL TRANSFER - LONG TERM FOLLOW-UP OF 50 PATIENTS

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Background. We investigated the use of donor-derived cytomegalovirus (CMV) specific cytotoxic T cells administered early post transplant to prevent CMV disease. **Methods.** CMV CTL were generated from CMVseropositive haemopoietic stem cell donors by three methods**.** Cohort 1: donor monocyte derived dendritic cells (mo-DC) from peripheral blood were used to present the CMV peptide NLVPMVATV to autologous donor T cells. Cohort 2: mo-DC transfected with an adenoviral vector containing the CMV pp65 gene (Adpp65) were used to stimulate autologous donor T cells. Cohort 3: as for cohort 2 but the stem cell harvest product was the source of effector T cells. A single cell infusion of $2x10^7$ cells/m^2 was administered to transplant recipients from day 28. Patients were assessed for infusional safety, transplantation outcomes and CMV directed immune function. **Results.** We infused 50 patients with CMV CTL between 2003 and 2011 (cohort 1 n=10; cohort 2 n=33, cohort 3 n=10). Indications for transplantation were AML (n=30; 17 in CR1, 11 in CR2 and 2 with persistent disease), ALL (n=7), MDS (n=3), NHL (n=5), Hodgkin's lymphoma (n=1), multiple myeloma (n=2) and non-malignant conditions (n=2). 20 patients received myeloablative conditioning and 30 received reduced intensity conditioning. 50% of patients received *in vivo* T cell depletion with anti-thymocyte globulin (n=22) or alemtuzumab (n=3). There were 36 sibling donors and 14 unrelated donors. 45/50 were fully HLA matched and 5/50 had 1 antigen mismatch. Donor/recipient CMV serostatus was pos/pos (n=36) and pos/neg (n=14). Median follow up was 26 months post transplant (range 2 to 80). 50% of patients reactivated CMV within 100 days post transplant (17/25 pre-infusion, 8/25 post infusion). There were no cases of CMV reactivation post day 100. Reactivation rates were not different from those observed in 168 historical controls at our institution. Median peak CMV titre was <600 copies/mL in reactivating patients (below the limit of linearity of the assay). Three patients (6%) required treatment with intravenous ganciclovir post T cell infusion (compared with 46% requiring CMV pharmacotherapy in controls). Only one patient developed CMV disease (pneumonitis) (compared with 9% CMV infection rate in controls). He died despite treatment with ganciclovir. 38% (19/50) developed acute GVHD (grade I-II 15/19, III-IV 4/19). Of these, 10 followed cell infusion (grade I-II 7/10, III-IV 3/10). Two patients (4%) died of acute GVHD post T cell infusion. Median follow-up was 18 months post transplant (range 2 to 80). At 5 years post transplant, progression free survival was 69% and overall survival was 70%. **Conclusions.** In this non randomised study, adoptive transfer of CMV specific CTLs appeared to reduce the requirement for CMV specific pharmacotherapy with ganciclovir or foscarnet without affecting the rate of CMV reactivation. A low rate of CMV disease was observed. We postulate that infused cells are able to limit viral reactivation and prevent progression to tissue infection.

0090

RAPID ANALYSIS AND SELECTION OF MINOR HISTOCOMPATIBILITY ANTIGENS WITH THERAPEUTIC RELEVANCE BY MICROARRAY GENE EXPRESSION ANALYSIS

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Background. Patients with hematological malignancies can be successfully treated with allogeneic hematopoietic stem cell transplantation. Beneficial Graft-versus-Leukemia (GvL) reactivity, however, is often accompanied with undesired Graft-versus-Host Disease (GvHD). GvL and GvHD are mediated by donor T-cells recognizing minor histocompatibility antigens (MiHA). Donor T-cells recognizing ubiquitously expressed MiHA may induce GvL as well as GvHD, whereas MiHA with hematopoietic restricted expression may be selectively targeted in GvL. We previously developed a method based on whole genome association scanning for high throughput discovery of novel MiHA, and new strategies are now required for rapid analysis and selection of MiHA with therapeutic relevance. **Aims.** The aim of the study was to investigate

whether MiHA with therapeutic relevance can be rapidly selected based on cell type specific expression patterns as determined by microarray gene expression analysis. **Methods.** Malignant and non-malignant hematopoietic cells of different origins were isolated by flowcytometry based on expression of specific markers. Non-hematopoietic cell types included isolated or cultured skin derived fibroblasts (FB) and keratinocytes, proximal tubular epithelial cells, hepatocytes and small intestine, colon and lung epithelial cells. Various non-hematopoietic cell types were also cultured in the presence of IFN-g to mimick the pro-inflammatory cytokine milieu of the early post-transplantation period. Total RNA was isolated and processed for gene expression profiling. T-cell recognition of primary (malignant) hematopoietic and (cytokine treated) non-hematopoietic cell types was measured by IFN-g ELISA. **Results.** The quality of the microarray gene expression database was validated by demonstrating expression of specific markers for each cell type, and confirming expression patterns of well-known hematopoietic restricted MiHA. Next, we used microarray gene expression analysis to investigate the cell type specific expression profiles of 12 MiHA as recently discovered by whole genome association scanning. T-cell experiments demonstrated that 7 of these 12 MiHA were recognized on skin derived FB, of which 6 MiHA were only recognized after pre-treatment with IFN-g. T-cell clones for the remaining 5 MiHA failed to recognize (cytokine treated) FB. Microarray expression analysis demonstrated that all genes encoding MiHA that are recognized on (cytokine treated) FB exhibited broad expression profiles. Of the 5 MiHA that were not recognized on (cytokine treated) FB, 3 genes showed ubiquitous expression, indicating that gene expression is required, but not always sufficient for T-cell recognition, and that other factors such as antigen processing and presentation and surface expression of adhesion and costimulatory factors also play a role. The genes encoding the remaining 2 MiHA displayed hematopoietic restricted expression patterns. These genes include ARHGDIB, which is expressed in a large variety of (malignant) hematopoietic cell types, and EBI3, which is selectively expressed on mature dendritic cells. T-cell experiments confirmed surface expression of the HLA-B*0702 restricted LB-ARHGDIB-1R on primary leukemic cells of different origins, illustrating the therapeutic relevance of this MiHA. **Summary and Conclusions.** Our data demonstrate that microarray gene expression analysis allows rapid analysis and selection of MiHA with therapeutic relevance for development of novel T-cell therapies with the aim to induce a more favorable balance between GvL/GvHD in alloSCT.

0091

HLA-CLASS II UPREGULATION DURING AN ONGOING VIRAL INFECTION CAN LEAD TO HLA-DP DIRECTED GRAFT-VERSUS-HOST DISEASE AFTER HLA-DPB1 MISMATCHED CD4+ DONOR LYMPHOCYTE INFUSION

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Background. In allogeneic hematopoietic stem cell transplantation (alloSCT), T-cell depletion of the graft prevents severe acute Graft-versus-Host Disease (GvHD), but impairs post-transplant anti-tumor and anti-viral immunity. Early intervention with donor lymphocyte infusion (DLI) after T-cell depleted alloSCT (TCD-alloSCT) is frequently associated with reintroduction of GvHD. However, administration of CD8+ T-cell depleted DLI has been associated with conversion to donor hematopoiesis, disease remissions in patients with relapsed malignancies and improved immune reconstitution against pathogens, in the absence of severe GvHD, providing a rationale for exploration of CD4+ T-cell based immunotherapy as treatment modality for hematological malignancies. AIMWe recently initiated a clinical study in which patients with hematological malignancies are treated with prophylactic CD4+ DLI at 3 months after HLA-matched unrelated donor TCD-alloSCT. Two patients with acute myeloid leukemia converted to full donor chimerism with concomitant Grade-1/2 skin and Grade-3/4 colonic acute GvHD after a single infusion of $0.5*10^6$ purified CD4+ T-cells/kg following 10/10 HLA-matched, but HLA-DPB1 mismatched, TCD-alloSCT. We performed detailed analysis of the clinical course and specificity of T-cell immune responses to investigate the immune mechanisms underlying development of the beneficial and detrimental clinical responses in these patients. **Methods.** Based on co-expression of HLA-DR, activated CD4+ and CD8+ T-cells were clonally isolated from peripheral blood samples obtained during GvHD after, and tested for recognition of patient- and donor-derived target cells using IFNg-ELISA. **Results.** From patients 1 and 2, we isolated in total 19 (2,5%) and 16 (5,6%) CD4+ T-cell clones, which were alloreactive as illustrated by specific recognition of patient-, but not donor-, derived hematopoietic cells. Blocking studies with specific monoclonal antibodies and retroviral transduction of donor EBV-LCL with patient mismatched HLA-DPB1 alleles confirmed allorecognition of patient HLA-DPB1 alleles by all alloreactive CD4+ T-cell clones. The majority of HLA-DPB1 specific CD4+ T-cell clones from both patients recognized patient-derived skin-fibroblasts expressing HLA-class II molecules induced by pre-treatment with pro-inflammatory cytokines. Alloreactivity was demonstrated for only one CD8+ T-cell clone (0,3%) from patient 2, which was restricted by HLA-C and failed to recognize patient-derived cytokine-treated skin-fibroblasts. At the time of CD4+ DLI, both patients were in complete remission and complete donor chimerism was shown in the B-cell and myeloid compartments, whereas predominantly patient chimerism (89-100% patient) was observed in the T-cell compartments. In both patients these residual patient-derived T-cells were activated as reflected by expression of HLA-class II, and most of these T-cells were CMV-specific T-cells as a consequence of CMV reactivations. Summary and Conclusions. Our data indicate a direct role for alloreactive HLA-DP directed CD4+ T-cells as mediators of profound clinical responses after prophylactic HLA-DPB1 mismatched CD4+ DLI. We postulate that (local) release of pro-inflammatory cytokines by patient-derived CMV-specific T-cells expanded as a consequence of CMV reactivation induced HLA-class II expression on non-hematopoietic cells, thereby making them targets for alloreactive HLA-DPB1 specific CD4+ T-cells in GvHD. In conclusion, our results suggest that active viral infections at the time of prophylactic CD4+ DLI play a relevant role in development of GvHD after HLA-class II mismatched TCD-alloSCT.

0092

DONOR T-CELLS SPECIFIC FOR HEMATOPOIETIC-RESTRICTED MINOR HISTOCOMPATIBILITY ANTIGENS ARE INDUCED IN PATIENTS IN WHICH GRAFT-VERSUS-LEUKEMIA REACTIVITY IS ACCOMPANIED WITH GRAFT-VERSUS-HOST DISEASE

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Background. Patients with hematological malignancies can be effectively treated with allogeneic hematopoietic stem cell transplantation (alloSCT) and donor lymphocyte infusion (DLI). In HLA-matched alloSCT, donor T-cells can mediate beneficial Graft-versus-Leukemia (GvL) reactivity due to recognition of minor histocompatibility antigens (MiHA) on patient malignant cells. MiHA are peptides in HLA-molecules that differ between patient and donor due to single nucleotide polymorphisms. Donor T-cells may also induce Graft-versus-Host Disease (GvHD) when MiHA with ubiquitous expression on hematopoietic and non-hematopoietic tissues are recognized. Development of broadly applicable T-cell therapies to selectively stimulate GvL reactivity after HLA-matched alloSCT without GvHD requires identification of multiple MiHA with hematopoietic-restricted expression in common HLA class I molecules.**Aim**The aim of the study was to analyze whether hematopoietic-restricted MiHA can be identified in patients with combined GvL and GvHD reactivity. **Methods.** Patient 1 with acute myeloid leukemia and patient 2 with chronic myeloid leukemia entered complete remission following DLI after HLA-matched alloSCT. In both patients, induction of GvL reactivity coincided with GvHD. Single CD8+ T-cells were isolated from peripheral blood obtained 6-9 weeks after DLI based on CD137 expression after 48 hours of stimulation with patient-derived EBV-B cells (patient 1) or CML cells *in vitro* modified into professional antigen presenting cells (CML-APC; patient 2). T-cell recognition of hematopoietic cells and (cytokine-treated) fibroblasts (FB) was measured by IFN-γ ELISA. MiHA were identified by whole genome association scanning (WGAs) and expression profiles were determined by microarray gene expression analysis using (malignant) hematopoietic and (cytokine-treated) non-hematopoietic cell types that are targeted in GvL and GvHD. **Results.** A total number of 399 T-cell clones were isolated from the two patients. All T-cell clones were tested for recognition of patient-derived EBV-B cells (patient 1) or CML-APC (patient 2) as well as donor EBV-B cells, and 170 T-cell clones were shown to be specific for MiHA. All MiHA specific T-cell clones were subsequently analyzed for reactivity against a mixture of peptides of previously identified MiHA. T-cell clones specific for LB-GEMIN4-1V and ZAPHIR were isolated from patient 1, and T-cell clones for LB-ADIR-IF and LRH-1 from patient 2. These MiHA are ubiquitously expressed, except for LRH-1 which has a hematopoietic-restricted expression profile. Finally, all MiHA specific T-cell clones were tested against patient-derived FB, and the majority of T-cell clones were shown to recognize these cells after pre-treatment with IFN-γ. We identified 5 novel MiHA by WGAs, which were all ubiquitously expressed as demonstrated by microarray gene expression analysis. However, a minority of T-cell clones showed no recognition of (cytokine-treated) FB, and are currently under investigation for identification of MiHA with potential therapeutic relevance. **Summary and Conclusions.** The data show that donor T-cells recognizing hematopoietic-restricted MiHA can be induced in patients with combined GvL and GvHD reactivity. Our strategy of efficient isolation of MiHA specific CD8+ T-cell clones followed by rapid identification and expression analysis of MiHA by WGAs and microarray gene expression analysis for T-cell clones which fail to recognize patient-derived (cytokine-treated) fibroblasts is therefore relevant for discovery of novel hematopoietic-restricted MiHA with therapeutic relevance.

0093

PRODUCTION OF LEUKEMIA-REACTIVE T-CELLS VIA A GOOD MANUFACTURING PRACTICE PROCEDURE FOR TREATMENT OF PATIENTS WITH ACUTE OR CHRONIC B-CELL MALIGNANCIES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Donor T-cells recognizing malignant cells of the recipient can mediate a potent therapeutic graft-versus-leukemia (GvL) effect. However, due to the broad repertoire of specificities, application of unselected donor T-cells at the moment of allogeneic stem cell transplantation (alloSCT) or by donor lymphocyte infusions (DLI) early after transplantation harbors a high intrinsic risk of inducing severe GvHD. Moreover, the poor immunogenicity of most leukemias may further limit the potential therapeutic effect of DLI. These limitations may be overcome by the adoptive transfer of in-vitro generated leukemia-reactive T-cells containing a selected population of donor T-cells with specificities skewed towards recognition of (malignant) hematopoietic cells of the patient.In this study, we developed a Good Manufacturing Practice (GMP) procedure to generate potent leukemia-reactive T-cells for adoptive transfer into patients with recurrent or persistent B-cell malignancies after alloSCT.Primary malignant B-cells harvested from peripheral blood (PB) or bone marrow (BM) of patients with chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), or mantle cell lymphoma (MCL) were transformed in-vitro into antigen presenting cells (APC) by crosslinking of CD40 using a 2-step strategy consisting of CpG/IL4 pre-activation of the malignant cells followed by co-culture with irradiated CD3/28 activated donor T-cells expressing CD40-ligand. Next, naïve donor T-cells enriched from the donor leukapheresis product depleted of CD14+ monocytes and CD25+ regulatory T-cells were stimulated with the malignant APC (T-cell/APC ratio 50/1) and cultured for 14 days in IMDM supplemented with heat-inactivated human serum, 10 ng/mL IL-7 and only 0.1 ng/mL IL-15. After a second stimulation with the malignant APC (T-cell/APC ratio 25/1) leukemia-reactive T-cells were isolated based on their expression of the activation marker CD137 using a clinical grade CD137-biotin reagent, anti-biotin beads and cliniMACS isolation (Miltenyi Biotec). Post-isolation culture was performed in IMDM supplemented with heat-inactivated human serum, 10 ng/mL IL-7 and 0.5 ng/mL IL-15 and irradiated donor PB mononuclear cells. The functional reactivity of the isolated T-cells against primary malignant B-cells, malignant APC and donor PBMC was tested at 7-10 days after isolation using flow-cytometry-based quantitative cytotoxicity assays and analysis of specific cytokine production. Sufficient numbers of phenotypical appropriate malignant APCs expressing CD40, CD80 and CD86 could be reproducibly generated from primary malignant B-cells both harvested at diagnosis or at a later stage after initial treatment. CD137+ fractions after cliniMACS isolation contained >50% CD137+ T-cells. Isolated leukemia-reactive T-cells contained an oligoclonal T-cell receptor (TCR) repertoire, as measured by analysis of TCR-beta chain expression, and showed profound cytotoxic activity (>50% lysis at 3/1 E/T ratio) against the primary malignant B-cells as well as against the malignant APC and no reactivity against donor cells. All T-cell products produced in the large scale testruns under GMP conditions met the pre-defined criteria for quality control and release for clinical application.We have developed a GMP-approved procedure for the generation of leukemia-reactive T-cells. A clinical phase I/II study is initiated to demonstrate the feasibility and safety of administration of these leukemia-reactive T-cells to patients with recurrent or persistent mature B-cell neoplasms after allo-SCT.

0094

A SUBSET OF NAIVE T-CELLS SHOWS HLA-RESTRICTED AUTO-REACTIVITY AGAINST UNPULSED AUTOLOGOUS DENDRITIC CELLS HAMPERING ENRICHMENT OF LOW-FREQUENCY ANTIGEN-SPECIFIC T-CELLS BY *IN VITRO* PRIMING

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BackgroundAdoptive transfer of in-vitro selected antigen-specific T-cells is an elegant strategy to treat persistent disease after allogeneic stem cell transplantation. In these cases, induction of primary immune responses from the naive donor T-cell repertoire is required. Priming of antigen-specific naive T-cells by in-vitro stimulation with peptide-loaded autologous monocyte-derived dendritic cells (autoDCs) has been shown to be feasible, but poorly reproducible. An observed unexpected proliferative response of naive T-cells following stimulation with autoDCs in the absence of exogenously loaded peptides (the 'autologous mixed lymphocyte response' (autoMLR)) hampers the enrichment of low-frequency antigen-specific T-cells.AimTo unravel the autoMLR phenomenon by investigating the frequency, nature, and specificity of naive T-cells responding to unpulsed autoDCs.MethodsPurified human naive T-cells (CD3+CD45RO-CD27+) were labeled with the fluorescent dye PKH and stimulated with unpulsed autoDCs (R/S ratio 1/1) in medium containing 5 ng/mL IL-7 and 0.1 ng/mL IL-15. Proliferation, as indicated by PKH dilution, was monitored 7-10 days after stimulation. Based on their proliferative response, autoDC-reactive (PKH-low) and non-reactive (PKH-high) T-cells were isolated single cell/well or in bulk by flow cytometric cell sorting. Secondary stimulations were performed with autologous or allogeneic HLA-matched and mismatched stimulator cells with or without HLA-blocking antibodies.ResultsThe autoDC-reactive T-cell population, comprising a polyclonal population of CD4+ and CD8+ T-cells, represented 1-3% of the total naive T-cell pool, which was similar in adult and cord blood-derived naive T-cells. The autoDC-reactive T-cells acquired CD45RO expression, while the non-reactive counterparts remained naive in response to stimulation with autoDCs, but could still respond to allogeneic stimulator cells. Increasing the number of autoDCs enhanced the proliferation rate of the autoDC-reactive T-cells without affecting the frequency of responding cells. Although both CD4+ and CD8+ naive T-cells showed autoDC reactivity, we could only generate CD4+ clones retaining their reactivity against autoDCs. The autoDC-reactive CD4+ clones produced IFN-γ and/or IL-4 and down-regulated their TCR/CD3 complex upon stimulation, but most T-cell clones were not cytotoxic against autoDCs. Interestingly, the autoDC-reactive T-cell clones showed profound reactivity against autologous monocytes, immature DCs, and primary myeloid leukemic cells (AML), but not against non-myeloid cells including EBV-LCL, PHA-blasts, and (activated) B-cells, indicating the myeloid-lineage specificity of the autoDC reactivity. Importantly, lack of reactivity against autologous EBV-LCL cultured in medium supplemented with the cytokine cocktail used for DC generation excluded the possibility of recognition of cytokines or medium components presented by stimulator cells. Analysis of reactivity against a panel of allogeneic DCs and primary AML cells matched for one or more of the HLA class II molecules and HLA blocking experiments revealed HLA-restriction of the autoDC reactivity. From individual donors both HLA-DP, -DQ and -DR restricted autoDC-reactive clones were isolated, further illustrating the polyclonality of this reactivity.ConclusionsIn conclusion, we demonstrated the existence of T-cells in the naive repertoire from healthy donors showing HLA-restricted auto-reactivity against autoDCs. These T-cells show myeloid lineage-specific reactivity against HLA-matched allogeneic cells. Removal of these autoDC-reactive T-cells is essential for in-vitro enrichment of low-frequency antigen-specific T-cells from the naive repertoire using antigen-loaded DCs as stimulator cells.

0095

DONOR LYMPHOCYTE INFUSIONS (DLI) CD3+ AFTER REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC STEMS CELLS TRANSPLANTATION; SINGLE CENTRE EXPERIENCE

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Donor lymphocyte infusions (DLI) can induce remission in patients with hematologic malignancies who relapse after allogeneic stem cells transplants (alloSCT). However Graft versus Host Disease (GvHD) remains the major complication of this strategy. We have been using escalating doses of DLI for many years, and wanted to assess risk factors for GvHD and transplant related mortality (TRM) as well as disease outcome according to reason of DLI.We analyzed 77 patients who received a total of 87 DLI, for different reasons and at different intervals from transplant. The median number of DLI was 2 (1-4) the median interval between transplant and DLI was 9 months (1-41), the median number of infused CD3+ cells /kg of recipients body weight was 2,8x10^7 ($1x10^6$- $11,8x10^7$). The diagnosis were multiple myeloma (n=25), acute leukemia (n=19), lymphoma (n=18), chronic myeloid leukemia (n=5), myelofibrosis (n=3), myelodysplastic syndrome (n=2), other diagnosis (n=5). Median patient age was 48 years (20-67). The cause of DLI was relapse or progression in 40 patients (52%), residual disease in 11 patients (14%), prophylactic in 13 patients (17%), and the presence of mixed chimerism in 13 patients (13%). Factors studied for an association with GvHD and TRM were donor type (siblings/alternative donors), year of DLI (</> 2006), maximum dose of DLI (</> $1x10^7$), recipient age (<=/>50 years), number of DLI (</> 1), interval transplant-DLI (</> 6 months), cause of DLI (relapse vs. prophylaxis vs. mixed chimerism vs. residual disease), disease type and recipient gender. Seven patients (9%) developed acute GvHD grade II-IV, and 5 patients (6%) developed an extensive chronic GvHD. In univariate analysis we could identify the interval transplant-DLI <= 6 months, the dose of DLI (>=$1x10^7$) and DLI number as a predictors factors of acute GvHD: In multivariate analysis this result was confirmed only for the interval transplant-DLI <= 6 months with HR=0.10 (0.02-0.51) (p=0.006). With a median follow up of 43 months (9-132), 43 patients are alive

(56%). The primary cause of death was relapse of the original disease in 29 patients (38%), whereas 4 died of TRM (5%). Only the interval transplant DLI <= 6 months was identified as a factor predicting TRM in univariate analysis. The overall survival (OS) at 5 years was 45% [CI 95% (34-56)]; the progression free survival (PFS) at 3 and 5 years was 24% [CI 95% (13-33)] and 17% [CI 95% (8-26)] respectively. PFS after DLI significantly differed according to reason of DLI. In this relatively large series of consecutive DLI, the risk of GvHD was relatively low, and we could identify only the interval transplant-DLI less than 6 months as significant predictor of acute GvHD and TRM. At a preliminary analysis, DLI seem to show efficacy when administered as prophylaxis, for mixed chimerism and residual disease but not for overt relapse. Our findings indicate that this form of adoptive immunotherapy is well tolerated and induces a low incidence of GvHD and TRM, supporting further investigation as an upfront modality to enhance graft versus tumor response in high risk patients.

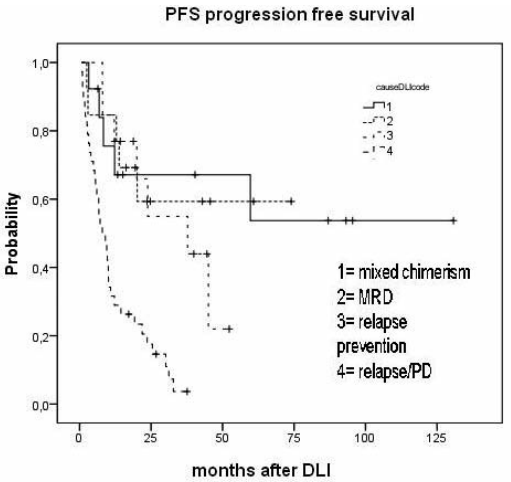

Figure 1. PFS.

0096

***IN VITRO* GENERATION OF CD4+CD25HI LYMPHOCYTES EXPRESSING HIGH LEVELS OF FOXP3 AND GITR FROM UMBILICAL CORD BLOOD NAÏVE T-CELLS**

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Background. The generation of induced regulatory T-cells (iTregs) *ex-vivo* holds promise in the clinics, in light of its potential immunotherapeutical use in conditions involving transplant rejection and autoimmune disorders. Nevertheless, reports from the literature are conflicting and are mainly derived from mouse or human peripheral blood. **Aims.** Evaluate the potential *in vitro* generation of iTregs from umbilical cord blood. **Methods.** CD4+CD45RA+ naïve cells were isolated from umbilical cord blood by negative selection and were activated with anti-human CD2/CD3/CD28 beads (1:2) in the presence of IL-2 (10, 50 or 100 U/ml) with (CD4med) or without combinations of TGF-beta (5 or 10 ng/ml) and atRA (50 or 100 nM) for 5 days. CD4+ gated lymphocytes were analyzed by flow cytometry, for the expression of CD25, FoxP3, TNFR2, GITR. CD4+CD25hi cells were sorted and tested for its ability to suppress proliferation of CD3+ cells (CFSE staining). Methylation analysis of FoxP3 TDSR region was also performed. **Results.** In the presence of atRA (100 nM), the percentage of CD25hi cells increased till the 3rd day, in all treatment conditions. In the 5th day, cell death occurred with low IL-2 levels (10U/mL), irrespective of TGF-beta concentration, while, at higher IL-2 levels (100U/ml), a higher concentration of TGF-beta (10 ng/ml) caused cell death. At optimal IL-2 levels (50U/ml), presence of atRA resulted in increased generation of CD25hi cells, reaching up to 30% of CD4+ cells, as compared to only 3% observed in the absence of atRA. Moreover, without atRA, cell death was noticeable at low TGF-beta concentration. Cells activated in the presence of 50 U/ml IL-2, 5ng TGF-B and 100mM atRA ($CD4_{TGF/atRA}$) showed the highest percentages of CD25hi T cells after 5 days of culture. From day 1 to day 5, the percentage of FoxP3+ cells in the CD25hi population increased in both conditions, but with higher percentages in $CD4_{TGF/atRA}$, reaching 99%; and only 50% in $CD4_{med}$. Importantly, while in $CD4_{med}$, FoxP3+ cells were almost absent in the CD25- population; in $CD4_{TGF/atRA}$ this percentage reached up to 55%. Similarly, in the 5th day, GITR+ cells were almost absent in $CD4_{med}$ in the CD25- population, while this percentage reached up to 32% in $CD4_{TGF/atRA}$. TNFR2 expression was restricted to CD25+ cells, with higher percentage of positive cells in the CD25hi population. Intriguingly, while TNFR2 was steadily expressed, in all days, in around 70% of the CD25hi cells in $CD4_{med}$; in $CD4_{TGF/atRA}$ this percentage decreased from 61% in day 1, to 9% in the 5th day. Finally, TDSR region was fully methylated and the suppressive capacity of CD4+CD25hi cells sorted from $CD4_{TGF/atRA}$ could not be observed. **Conclusions**. We show that high percentages of Foxp3+ CD4+CD25hi T-cells expressing GITR can be generated *in vitro*, from umbilical naïve T cells. Despite the high levels of Foxp3+, methylation of FoxP3 TDSR and inability to suppress proliferation of activated lymphocytes indicated that additional stimuli is necessary to drive full differentiation of CD4+ naïve cord blood cells into functionally capable regulatory T cells. **Support.** FAPESP and CNPq.

0097

MULTIPOTENT STROMAL CELLS INDUCE DIFFERENTIATION OF MONOCYTES TOWARDS AN IL-10 PRODUCING PHENOTYPE IN AN IL-6 DEPENDENT MANNER, AND PREVENT DIFFERENTIATION TOWARDS DENDRITIC CELLS

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Multipotent stromal cells (MSC) have been studied because of their immunomodulatory capacities, which make these cells interesting candidates for further clinical application. One of the proposed effects of MSC is the inhibition of dendritic cell differentiation. Both IL-6 and IL-10 interfere with the differentiation of monocytes to (immature) dendritic cells and skew the monocytes into the macrophage lineage. Since macrophages produce higher levels of IL-10 than monocytes, this induces an autocrine loop. We hypothesized that IL-6 and IL-10 may play a role in the inhibitory effect of MSC on the differentiation of monocyte towards dendritic cells.$CD14^+CD1a^-$ monocytes are cultured in RPMI containing 10% fetal calf serum, IL-4 (10 ng/mL) and GM-CSF (5 ng/mL) in the presence or absence of culture-expanded bone marrow derived MSC. Significantly lower numbers of $CD14^-CD1a^+$ immature dendritic cells (iDC) were generated in cultures containing MSC than in control cultures without MSC (15.1% versus 70.6%, $p < 0.01$). Similar results were obtained in cultures that contained MSC-conditioned medium. In co-cultures with allogeneic $CD4^+CD25^-$ T cells, iDC ($CD14^-CD1a^+$) displayed a higher allostimulatory capacity than $CD14^+CD1a^-$ cells derived from MSC-containing cultures. Conditioned media from co-cultures of monocytes and MSC contained higher levels of IL-6 and IL-10, in comparison with cultures without MSC (IL6: 221.6 pg/ml versus 11.9 pg/ml, $p < 0.001$, $n = 6$; IL-10: 984.6 pg/ml versus 333.4 pg/ml, $p < 0.01$, $n = 4$). We showed that IL-10 is produced by the monocytes in these cultures and not by MSC. In cultures containing saturating concentrations of neutralizing antibodies to IL-10, no effect on the MSC-induced inhibition of monocyte-to-iDC differentiation was observed. In contrast, this process was significantly inhibited by neutralizing antibodies to IL-6. The differentiation of monocyte-to-iDC could be inhibited by both IL-6 and IL-10. IL-6 was constitutively expressed at high levels by MSC and this factor was responsible for the enhanced IL-10 expression and secretion by monocytes.We conclude that i) differentiation of monocytes towards iDC is inhibited by MSC through the production of IL-6 and ii) IL-6 induces the formation of IL-10 producing monocytes, which exhibit a reduced allostimulatory capacity. Our results indicate a crucial role for monocytes in the immunomodulatory properties of MSC.s.m.melief @lumc.nl

0098

MULTIPOTENT STROMAL CELLS MODULATE MONOCYTES TO INDUCE THE GENERATION OF CD4+CD25HIFOXP3+ REGULATORY T CELLS

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Multipotent stromal cells (MSC) have been shown to possess immunomodulatory capacities and are therefore explored as novel cellular therapy. In this study we investigated the role of MSC in the generation of regulatory T cells. We focused on the cellular interactions and secreted factors that are essential in this process.In an *in vitro* culture system (RPMI containing 10% fetal calf serum) of PBMC we showed that significantly more $CD4^+CD25^{hi}FoxP3^+$ T cells (Tregs) were generated in the presence of culture-expanded bone marrow derived MSC than in cultures without MSC (21.0% versus 2.7%, $p < 0.001$; n=5). Similar results were obtained with MSC-conditioned medium, indicating that this process is dependent on soluble factors secreted by the MSC. The

MSC-induced PBMC, containing Tregs, were functionally suppressive since they were able to suppress the proliferation of $CD4^+CD25^-$ T cells stimulated with αCD3/αCD28 beads. Removal of monocytes from the PBMC by MACS or adherence, completely prevented the generation of Tregs. This shows that the interaction between MSC and monocytes is essential for generating the population of Tregs. We showed that MSC enhanced the survival of monocytes within the PBMC population and induced skewing of the monocytes into a M2-macrophage-like phenotype. The generation of Tregs by MSC was significantly suppressed by addition of neutralizing antibodies directed against the TGF-β1 receptor but not by anti-IFNγ antibodies. These results indicate that TGF-β1, but not IFNγ, is involved in this process. Since TGF-β1 is constitutively secreted by MSC, it is conceivable that the MSC-induced generation of Tregs is mediated by TGF-β1 and that TGF-β1 secretion is independent of the interaction between MSC and PBMC. Experiments with inhibitors of heme oxygenase 1 (SnPP) and prostaglandin E2 (indomethacin) did not reveal a significant contribution to the MSC-induced generation of Tregs.In summary, we report that i) MSC promote the generation of regulatory T cells and ii) the induction of Tregs requires the presence of monocytes and of TGF-β1.s.m.melief@lumc.nl

0099

EFFECTIVENESS OF WT1 PEPTIDE VACCINATION IN COMBINATION WITH IMATINIB THERAPY FOR AN IMATINIB-RESISTANT CML PATIENT

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Background. Although tyrosine kinase inhibitors (TKIs) have brought a dramatic improvement of the prognosis of CML, the extermination of CML stem cells is not yet achieved. It is well known that the induction and long-term maintenance of CTLs is inevitable for the eradication of cancer stem cells or MRD. WT1 is highly expressed in various types of leukemia including CML and WT1 protein is one of the most promising leukemia associated antigens for induction of CTLs. CML in CP in which the amount of CML cells was decreased remarkably by TKI is an ideal phase for the examination of effectiveness of immunothearpy. Therefore, in order to clarify the safety and effectiveness of WT1 peptide vaccination for the patients with CML, we started WT1 peptide vaccination in combination with imatinib therapy for a patient with CML. We could confirm the suppression of residual CML cells and the obvious existence of WT1 specific CTLs still over 3 years after discontinuation of vaccination. **Materials and Methods.** A 51 years-old male with CML in CP had been treated with 400 mg imatinib for 4 years. bcr-abl transcripts decreased transiently but gradually increased to more than 1,000 copies thereafter. HLA-A*2402-restricted 9mer WT1 peptides (CYTWNQMNL; a.a. 235-243), which had been identified to possess an anti-tumor immunogenicity, were administered subcutaneously at the dose of 1 mg/day every 2 weeks in combination with 400 mg imatinib for first 5 months and thereafter every 4 weeks for 12 months. The vaccination was undertaken 22 times totally. This protocol had been approved by ethical committee of Niigata University. The appearance of WT1-specific CTLs in PB was confirmed by evaluating the frequency of MHC/WT1 $tetramer^+CD8^+$ T cells by using mixed lymphocyte peptide culture (MLPC). **Results.** Although bcr-abl transcripts increased up to more than 2,000 copies after the the initiation of WT1 vaccination every 2 weeks, the transcripts have decreased to less than 500 copies by the administration of WT1 peptides every 4 weeks. After seven months from the cessation of WT1 peptide vaccination bcr-abl transcripts decreased to the level of major molecular response (MMR), which is lasting thereafter for 37 months. While WT1-specific CTLs were not detected in PB before WT1 peptide vaccination, the CTLs appeared after the second vaccination and remained at the level of nearly $15/10^6$ $CD8^+$ T cells thereafter. The MHC/WT1 $tetramer^+$ cells showed cytotoxicity against only the cells expressing WT1 and HLA-A*2402. Because the CML cells have not increased in molecular level after the cessation of vaccination, change of the treatment from imatinib to second-generation TKI is not planned now. **Conclusions.** WT1 peptide vaccination for an imatinib-pretreated CML patient is feasible and effective, which is due to the long-lasting amplification of WT1-specific CTLs with cytotoxicity against WT1-expressing leukemia cells. Tumor antigen specific peptide vaccine therapy in combination with molecular targeted therapy is one of the potent methods for eradicating leukemic stem cells.

0100

LARGE SCALE SCREENING OF CD8+ AND CD4+ T CELL EPITOPES IN THE IMMUNODOMINANT CMV PROTEINS IE1 AND IE2

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Background. Cytomegalovirus (CMV) is an important human pathogen in immunocompromised hosts causing severe disease in hematopoietic cell and solid organ transplant recipients. Both CD4+ and CD8+ T cells are important for long-term control of the virus. Identification of CMV specific T cell epitopes has primarily focused on CD8+ T cell epitopes. **Aims.** To characterize the repertoire of CMV-specific CD4+ and CD8+ T cell responses against immediate early antigen 1 and 2 (IE1 and IE2) in healthy CMV+ donors. **Methods.** Using intracellular cytokine secretion (ICS) and IFN-γ ELISPOT assays, PBMCs from CMV+ donors were screened for recognition of overlapping 15mer peptides spanning the entire IE1 and IE2 proteins. To identify CD8+ T cell epitopes, the optimal peptide length and HLA-class I restriction of the 15mer peptides were predicted using the predictor, HLArestrictor, and validated by tetramer staining. To identify CD4+ T cell epitopes, peptide-HLA class II affinity measurements guided the production of HLA class II tetramers, and these were used to validate CD4+ T cell epitopes. **Results.** We have identified and validated 12 CD8+ T cell epitopes (5 novel) representing HLA-A, -B, and -C alleles. We have also identified 29 HLA class II restricted CD4+ T cell epitopes (20 novel) and validated 7 of these with HLA class II tetramers. **Conclusions.** We have found multiple novel HLA class I and II T cell epitopes in IE1 and IE2. In IE1, we found both CD4 and CD8 epitopes, whereas CD4 epitopes dominated in IE2. In general, each individual donor recognized more CD4 epitopes than CD8 epitopes, but the frequencies of CD8+ T cells were often higher than the frequencies of CD4+ T cells. Interestingly we have found 5 dominant CD4 epitopes that were recognized by many donors and restricted to several different HLA class II molecules. These could be promising candidates for T cell targeted vaccine development and adoptive T cell transfer.

Hematopoietic stem cells and microenvironment 1

0101

IDENTIFICATION OF OSTEOBLAST STIMULATING FACTOR-5 AS A NOVEL REGULATOR OF EARLY B LYMPHOCYTE DEVELOPMENT

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Background. Early lymphocyte development is highly dependent on complex interactions within bone marrow microenvironment. Although the involvement of several categories of proteins has been reported, evaluating roles of individual stromal products in lymphocyte regulation is still informative to understand normal/malignant proliferation and differentiation of lymphocytes. **Aims.** We aimed to identify new key players to regulate lymphocyte development *in vivo*.Methods and **Results.** As the first screening, we comprehensively identified cell surface or secreted molecules produced by MS-5 stromal cells with signal trap **Methods.** The 5'-portion of MS-5-derived cDNA fragments were cloned into a HPC4-TF/pEFBOS vector, which produces chimeric proteins composed of cDNA-proteins, a HPC4-epitope tag, and tissue factor transmembrane. Cell surface expression of the chimeric proteins was detected by reactivity against anti-HPC4 antibodies with flow cytometory. The cDNA library was transfected into COS7 cells, and then cells expressing a HPC4-epitope on their surface were collected by cell sorting, followed by recovery of plasmids from the sorted cells. After three-time enrichment of cDNAs carrying signal sequences, the recovered plasmids were transfected into E. coli, and then 5,000 individual colonies were picked and stored. Based on HPC4-antibody recognition, 21 kinds of proteins carrying signal peptides, such as osteopontin, proliferin, osteoblast stimulating factor (OSF)-1, OSF-5, pre PDGF receptor, collagens, and 2 unknown proteins were identified. As the second screening, we selected proliferin, OSF-1, and OSF-5 by northern blots showing dominant expression on stromal cells and by information about expression in hematopoitic tissues and relationship with proliferation or differentiation. As the third screening, we generated transgenic chimera (Tg) mice, which produce these three molecules respectively under control of the immunoglobulin-kappa chain promoter to investigate *in vivo* effects. Tg mice are characterized by expression of proposal proteins in mature B cells without any influence on ontogeny. OSF-5-Tg mice showed significantly decreased number of blood cells in peripheral blood, spleen, and bone marrow as compared with control mice while either OSF-1- or proliferin-Tg mice did not. Flow cytometry of lympho-hematopoietic organs revealed that all Tg mice showed normal patterns of staining for T cells (CD4 and CD8 in thymus; CD3 in spleen) and myeloid cells (Mac1 in BM). In B cells, only OSF-5-Tg mice showed great decrease of $B220^+$ cells in peripheral blood, spleen, and bone marrow. OSF-5-Tg bone marrow cells have normal number of hematopoietic stem ($lineage^-Sca\text{-}1^{high}c\text{-}kit^{high}$) and pro-B ($CD43^{high}B220^+$) cells, but very few pre-B ($CD43^{dull}B220^+$) and immature B ($CD43^-B220^+$) cells. Thus, OSF-5 is likely to be a novel regulator of B lymphocyte development *in vivo*. OSF-5 is known to have two splicing variants, a secreted variant 1 and an intracellular variant 2. Our RT-PCR analysis revealed that OSF-5 variant 1 was expressed by stromal cells and OSF-5 variant2 by hematopoietic cells although OSF-5 mRNA was detected in a variety of organs. We have established *in vitro* culture systems using early lymphocyte progenitors and are now investigating roles of each OSF-5 variant. **Conclusions.** We successfully identified OSF-5 as a novel bone marrow component, which preferentially regulates early B lymphocyte development.

0102

RESOLUTION OF NUCLEAR FACTOR I-A CHROMATIN SIGNATURE BY POLYCOMB AND MICRORNA PROMOTER TARGETING ACTIVITIES DIRECTS HUMAN GRANULOPOIESIS

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Background. Stem/progenitors lineage commitment is accompanied by chromatin modifications at gene promoter "bivalent domains"with overlapping repressive H3K27me3 and activating H3K4me3 marks. This bivalency postpones cell commitment and, contemporaneously, maintains progenitor cells primed for alternate lineage fates. Polycomb (PcGs) and trithorax (TrxGs) proteins are responsible for the tri-methylation of H3K27 and H3K4, respectively. PcGs also possess RNA binding properties. Non-coding RNAs can be required for PcG recruitment to DNA, promoting chromatin remodeling and transcriptional gene silencing. The non-coding RNA family includes microRNAs (miRs), mainly mediating post-transcriptional gene silencing through limited base-pairing to complementary mRNA sequences. We previously found miR-223 functionally involved in a regulatory circuitry directing human granulopoiesis and identified Nuclear factor I-A (NFI-A) as a post-transcriptional miR-223 target and as a regulator of human hematopoietic stem cell/progenitor (HSC/HPCs) lineage choice. Up-regulation of NFI-A levels induces differentiation along the erythroid lineage while its down-regulation shifts HSC/HPCs fate towards the granulocytic lineage. **Aims.** To investigate whether epigenetic modifications affecting genes controlling stem cell identity and lineage choice could be responsible for the NFI-A gene silencing during maturation/differentiation of myeloid progenitors and the role of miR-223 in these events. **Methods.** The cellular models were: i) bone marrow, peripheral blood and CD34+-HSC/HPC, from informed healthy donors; ii) primary acute leukemia blasts from 20 patients; iii)HL60, HL60R, K562, NB4, NB4-MR4 cell lines in their wild-type form, ectopically expressing miR-223 or knocked out for miR-223 or Suz12, Dicer1, Ago1. NFI-A and miR-223 gene expressions were tested by qRT-PCR. Cell differentiation was investigated by morphological and immunophenotipical assays. MiR-223 association with the PcGs-RNAi complex, nuclear localization and NFI-A promoter targeting activity were visualized by confocal microscopy, co-immunoprecipitation and chromatin immunoprecipitation (ChIP) assays. ChIP assay using H3K27me3 and H3K4me3 antibodies revealed chromatin status on NFI-A promoter, whereas bisulphite sequencing addressed NFI-A promoter methylation. **Results.** We found that during human granulopoiesis miR-223 localizes inside the nucleus and locates on its complementary sequences present on NFI-A promoter near binding sites of PcG members YY1 and Suz12. The *in vivo* formation of a nuclear repressive complex, composed by miR-223 and YY1-Dicer-1 at YY-1 binding sites on NFI-A promoter, induces NFI-A transcriptional gene silencing through the resolution of promoter chromatin bivalent domains and heterochromatin formation. The integrity of evolutionarily conserved miR-223 complementary seed-matches on NFI-A promoter is required for NFI-A transcriptional gene silencing. Interestingly, the ectopic expression of miR-223 in human myeloid progenitors channels granulopoiesis and induces heterochromatic silencing of NFI-A gene, whereas miR-223 silencing produces the opposite effects. **Summary and Conclusions.** Our work identifies a novel cooperative pathway of site-specific regulation of gene expression acting on the NFI-A gene promoter triggered by the promoter recognition and transcriptional targeting activity by miR-223 and PcGs (YY1 and Suz12), which is relevant for HSC/HPCs granulocytic lineage determination. Thus, besides regulating translation of their mRNA targets, endogenous miRs affect gene expression at the transcriptional level, functioning at a critical interface between chromatin remodeling complexes and the genome to direct cell fate lineage determination of human hematopoietic cells.

0103

PERTURBATION OF FETAL LIVER HAEMATOPOIETIC STEM AND PROGENITOR CELL DEVELOPMENT BY TRISOMY 21

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Background. Constitutional trisomy 21 (T21) causes Down syndrome (DS). There is a striking increase in childhood acute leukaemia in DS; both myeloid (ML-DS) and B-lymphoblastic (B-ALL). DS leukaemias display distinct characteristics which support a crucial role for T21 in their pathogenesis, and for ML-DS at least, there is evidence to suggest initiation in fetal life. We have previ-

ously reported that by the second trimester, the T21 fetal liver (FL) myeloid progenitor compartment is abnormal and in addition B lymphopoiesis is impaired. We hypothesized that perturbation of FL HSC or multipotent progenitor proliferation and lineage-commitment may be responsible for these defects in FL myelo- and lymphopoiesis. **Aims.** To determine the effects of T21 on FL HSC and multipotent progenitors (MPP and LMPP) **Methods.** We performed detailed immunophenotypic analysis, clonogenic and lymphoid differentiation cultures and gene expression studies of HSC/MPP/LMPP and committed myeloid and B-lymphoid compartments of second trimester human T21 FL and compared these with normal FL of the same gestation. **Results.** Compared to normal FL (n=13), the frequency of HSC in T21 FL (n=8) was increased (T21: 7.8±0.9 vs. normal: 2.3±0.4%;p=0.0026) as was the frequency of MEP (T21: 46.3 ± 7.6 vs. normal: 18.0 ± 2.0%; p=0.005) whereas MPP and GMP were reduced (T21 vs. normal: 3.7±0.4% vs. 5.7±0.7%; p=0.0484 and 7.0 ± 2.9% vs.14.3 ± 1.0%; p=0.036 respectively). Although the frequencies of lymphoid primed multipotent progenitors (LMPP) and early lymphoid progenitors (ELP) were comparable; there was a marked reduction in the CD34+CD19+ committed B progenitors (CBP) in T21 compared to normal FL (T21: 3.1±1.3%vs.normal: 10.7±1.3%; p=0.0026), suggesting a block to B-lymphoid differentiation. *In vitro* clonogenic assays of flow-sorted HSC and progenitors showed increased clonogenicity of T21HSC (5-fold p<0.01), CMP (2.7-fold p<0.05) and MEP (2.5-fold p<0.05) compared to normal FL. Lineage output from T21 HSC, MPP, CMP and MEP showed marked skewing towards the megakaryocyte-erythroid lineage with increased megakaryocyte, megakaryocyte-erythroid and erythroid blast colonies. Although normal FL HSC, LMPP and ELP generated CD34-CD19+ B cells in MS5 co-cultures, their T21 FL counterparts demonstrated marked impairment in B-cell differentiation with very few CD34-CD19+ cells generated in MS5 co-cultures. T21 FL HSC expressed a multilineage gene expression programme but showed marked reduction in expression of early lymphoid genes (*FLT3, ETS1, MEF2C, NOTCH1* and *HES1)* compared to normal FL HSC. T21 FL LMPP, ELP and CBP also failed to upregulate early lymphoid genes (*FLT3*, *NOTCH1*, *IKAROS* and *RUNX1)* and this was accompanied by reduced expression of several key B-lymphoid genes (*EBF, IGH@, CRLF2, IL7R*A) compared to normal FL. **Conclusions.** We report the first evidence in primary human FL cells that T21 causes perturbation of immunophenotypically-defined HSC/MPP/LMPP. Expansion of HSC is accompanied by megakaryocyte-erythroid bias and extensive dysregulation of B-cell development. Therefore, in T21 FL, multiple defects in the HSC compartment with downstream effects on the committed myeloid and B-lymphoid progenitor compartments, may underlie the increased susceptibility of T21 haematopoietic cells to both myeloid and lymphoid leukaemic transformation.

0104

IMMUNE DEFECT AND VISUALIZATION OF CHROMOSOME 14 BREAKS AND TRANSLOCATIONS INVOLVING TCR A/D LOCI DURING EARLY T-CELL DEVELOPMENT LACKING ATM

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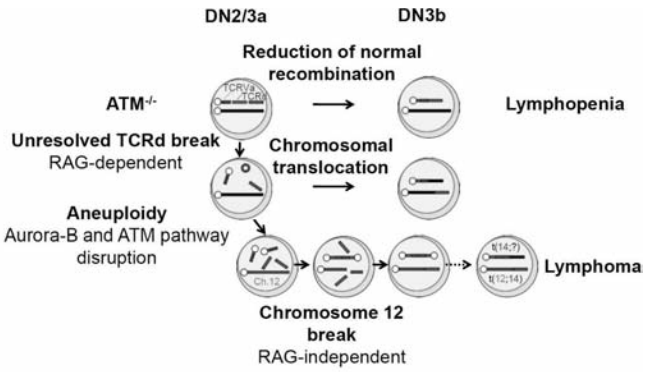
Background. Ataxia telangiectagia mutated (ATM) protein localizes at RAG mediated DNA DSBs and stabilizes DNA ends during V(D)J recombination. ATM deficient ($ATM^{-/-}$) mice are known to be predisposed to T-cell lymphopenia and T-cell lymphoma often associated with chromosome 14 translocation involving TCRd loci. $ATM^{-/-}$ mouse reportedly has a failure of T-cell development from double positive (DP) to single positive (SP) differentiation due to inefficient T-cell receptor a (TCRa) recombination. However, biological features of T-cell development at double negative (DN) phase and the timing of chromosome 14 translocation formation in $ATM^{-/-}$ mice have not been fully elucidated. **Aims.** We tried to understand how T-cell differentiation fails in thymocytes lacking ATM. Additionally, we tried to visualize when clomosomal breaks and translocations are generated during thymocytes development. **Methods.** We employed *in vitro* T-cell differentiation assay for obtaining metaphase spread from DN phase thymocytes. BM-progenitors were cultured on OP9-DLL1 cells with high-dose cytokine including Flt3-L, IL-7 and SCF. This culture condition halts T-cell differentiation at DN2-3a phase just before b-selection. Then, by reducing the concentration of Flt3-L and IL7, the differentiation arrest was released, leading to differentiation of thymocytes from DN3a to DN3b. **Results.** We demonstrate that $ATM^{-/-}$ thymocytes show a developmental failure at the transition phase from DN3a to DN3b in both ab and gd-lineages *in-vivo*. Consistent with *in-vivo* profiles, bone marrow (BM) progenitors of $ATM^{-/-}$ mice cultured with OP9-DLL1 show a delay at b-selection checkpoint in chronological order. This transitional failure was due to recombination failure of TCRb or d loci. We attempted to monitor when chromosome 14 translocation involving TCRa/d locus is generated during DN phase by using *in-vitro* culture system. Chromosome 14 breaks at TCRa/d locus were shown in 5% of $ATM^{-/-}$ DN2-3a cells, and these breaks persisted in 2.5% of $ATM^{-/-}$ DN3b-4 cells. Chromosome 14 translocation involving TCRa/d locus was detected in 0.8% of ATM-/- DN2-3a cells. The frequency of chromosome 14 translocation increased up to 11% of $ATM^{-/-}$ DN3b-4 cells. Chromosome 14 breaks and translocations were not detected in WT, $RAG2^{-/-}ATM^{+/-}$ nor $RAG2^{-/-}ATM^{-/-}$ thymocytes. These results suggest that in ATM deficient thymocytes generation of chromosome 14 breaks and translocations involving TCRa/d locus depend on RAG proteins, which mediate DNA DSB during b and gd selection. Additionally aneuploidy and dicentric chromosome 14 (dic(14:14)) with TCRVa amplification as well as sister chromatid breaks and translocations of chromosome 12 (t (12:14)) were also observed in minor population of $ATM^{-/-}$ cells. These snapshots might capitulate the RAG independent process for TCRVa amplification by breakage fusion breakage cycle and formation of recurrent chromosomal translocation toward thymic lymphoma in ATM deficiency. **Summary and Conclusions.** Our findings provide the first evidence that T-lymphopenia and chromosomal translocations in ATM deficiency derive from shared events involving a defect of repair of Rag dependent DNA breaks during early T-cell development. For lymphomagenesis Rag independent chromosomal instability processes are additionally involved in ATM deficiency.



Model for early thymocytes development lacking ATM
Schematic illustration for the impaired thymocyte development and creation of a translocation involving the TCR locus.

Figure 1.

0105

DEFICITS IN THE NF-KB ALTERNATIVE PATHWAY RESULT IN BOTH HEMATOPIETIC STEM CELLS AND MARROW STROMA FUNCTIONAL DEFECTS

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Background. The activation of NF-κB-inducing kinase (NIK) is generally known as the alternative (or non-canonical) NF-κB pathway, and drives the post-translational processing of p100 to mature p52, which results in the translocation to the nucleus of p52-containing complexes such as p52/RelB. The role of the alternative NF-κB pathway in the biology of hematopoietic stem cells (HSCs) is not known. The functions of HSCs in steady-state and under stress are controlled by a variety of molecules, which may provide different contribution to each process. The marrow microenvironment has a well established role in modulating the functions of HSCs. **Aims.** To study the functional capacities of HSCs and marrow stroma in mice deficient for two components of the alternative NF-κB pathway: NIK or p52. **Methods.** We studied HSCs and marrow stroma separately. The more immature hematopoietic compartment of marrow cells was characterised by flow cytometry. The amount of clonogenic progenitor cells in the marrow was assessed in standard CFU-GM cultures. The proliferative capacity of HSCs was assessed in *in vitro* liquid cultures with cytokines. The functional capacity of HSC was assessed *in vivo* in competitive repopulation assays. In vitro marrow stromal layers were generated from total bone marrow cells. We tested the ability of these stromal cultures for maintaining HSC function by seeding irradiated stromal layers with wild type HSCs. **Results.** The proportions of marrow cells with the immunophenotype of HSCs (lineage-depleted Sca1-positive ckit-positive cells, LSK, or CD150 positive CD48 negative cells) in either NIK-deficient or p52-deficient mice were similar to those in control mice. We found no differences in CFU-GM numbers in any of the mice

studied. Liquid cultures started with NIK-deficient marrow cells produced significantly less numbers of cells and CFU-GM, compared with those started with wild type marrow. NIK- or p52-deficient HSCs repopulated the B-, T- and myeloid-lineages but at significantly lower levels when compared to wild type HSCs. Total donor CD45.2 cells and total CD45.2 LSK cells were also significantly lower in the marrows of mice transplanted with NIK- or p52-deficient HSCs versus those of controls. When studying the effects of NF-κB alternative pathway defects on stromal cells, we observed that the stroma formation was delayed compared with that of control. The numbers of wild type CFU-GMs recovered 4 weeks after seeding were significantly lower in the NF-κB-deficient stromal cultures compared to those of controls.**Conclusions.** These results suggest that the NF-κB alternative pathway plays a role in the function of HSC that may be important under stress conditions. The pathway is also involved in the function of the hematopoietic stroma.

0106

BONE MARROW CD14+ MONOCYTES AND MACROPHAGES POLARIZATION TOWARD M2 PHENOTYPE PROMOTE MULTIPLE MYELOMA TUMOROGENICITY VIA THE CXCR4/CXCL12 AXIS - A NOVEL POTENTIAL PATHWAY FOR TARGTED THERAPY

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Background. Multiple myeloma (MM) is B-cell malignancy characterized by proliferation of plasma cells in the bone marrow (BM). Interaction between MM and BM microenvironment cells is critical for homing, growth and chemotherapy resistance of the tumor cells. Research to date has largely focused on the role of BM stromal cells (BMSCs) in MM progression. However, monocytes/macrophages may also contribute to MM growth. Macrophages are frequently found to infiltrate tumors. Tumor-associated macrophages (TAMs) exhibit predominantly M2-like phenotype and have been linked to diverse tumor-supporting activities. Elucidating the mechanisms involved in MM-macrophages interactions may have novel therapeutic applications. **Results.** MM cells specifically attracted peripheral-blood CD14+ monocytes. Interaction of MM with BMSCs significantly increased the ability of MM to recruit monocytes ($p<0.01$). We identified CXCL12 chemokine, produced by both MM and BMSCs, as critical regulator of CXCR4-positive monocytes migration. CXCL12 production was up-regulated in MM-BMSCs co-culture conditions, whereas blockage with anti-CXCR4 neutralizing antibodies significantly abrogated monocyte recruitment toward MM-derived conditioned medium ($p<0.01$). Furthermore, analyzing MM BM biopsies, we detected elevated levels of CXCL12 in comparison to normal BM samples, whereas malignant MM cells often represented the source of increased CXCL12 in BM. Next, the functional consequence of MM-macrophage interactions was examined. We showed that macrophages effectively induced MM proliferation and protected malignant cells from chemotherapy-induced apoptosis. Moreover, incubation with macrophages strongly induced the expression of various factors in MM cells, including chemokines CXCL12, CCL2 and CCL4, pro-inflammatory cytokine IL-1β and pro-angiogenic factors IL-8 and VEGF. Importantly, IL-10 secretion by MM cells was significantly up-regulated following the interaction with macrophages or BMSCs. These results suggest the possible role of MM cells in macrophage education and polarization. To further explore the modulatory effect of MM on macrophage polarization, we tested the expression of various M2 markers by macrophages following their interaction with MM cells. We found, that expression of M2-related chemokine CCL17, chemokine receptors CXCR1 and CXCR2 and scavenger receptor CD206 were up-regulated in macrophages following interaction with MM cells. Furthermore, MM cells fully blocked LPS-induced TNFa secretion from macrophages (a hallmark of M1 response), but further increased IL-10 secretion. Moreover, we analyzed the presence of M2-type macrophages in BM samples of MM patients. We detected increased numbers of CXCR4-expressing CD163+ CD206+ macrophages in MM BM (n=18) comparing to normal specimens (n=5). Finally, to explore the role of CXCR4/CXCL12 pathway in MM-mediated macrophage recruitment *in vivo*, a xenograft model of peritoneal macrophage migration was established. NOD/SCID mice were injected i.p. with CXCL12-expressing MM cell line RPMI8266 and F4/80+ macrophage recruitment into peritoneal cavity was measured. Injection of MM cells strongly increased the number of migrated macrophages, whereas CXCR4 blockade with neutralizing antibodies significantly suppressed macrophage migration. **Conclusions.** Taken together, these results identify macrophages as important players in MM tumorogenicity, demonstrate the role of MM in macrophage recruitment and M2 polarization and recognize CXCR4/CXCL12 axis as critical regulator of MM-stroma interactions and microenvironment formation. Our data provide the basis for future targeting MM-BMSCs and MM-macrophage interactions with anti-CXCR4 agents as therapeutic strategy in MM.

0107

QUANTITATIVE BUT NOT QUALITATIVE IMPAIRMENT OF MATURE FETAL B CELL DEVELOPMENT IN DOWN SYNDROME IS CELL-INTRINSIC AND ASSOCIATED WITH A MATURATION BLOCK AT THE PRO-B LEVEL

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Background. Children with Down syndrome (DS) have lymphopenia associated with an increased risk of autoimmune disease and susceptibility to infections. In conjunction with an increased risk of B acute lymphoblastic leukaemia, these observations suggest that trisomy 21 (T21) may specifically perturb B-lineage development. Consistent with this, we have recently found that in second trimester DS fetal liver (FL), early B cell development is characterised by a marked reduction in the frequency of Pre-proB and Pro-B committed B-progenitors suggesting that T21 impairs B-lymphoid development early in fetal life. Whether the effects of T21 are confined to the progenitor compartment or also affect mature B cell function and development in fetal life is unknown. **Aims.** To investigate mature B cell development in T21 FL, fetal bone marrow (FBM) and cord blood (CB). **Methods.** Frequency and immunophenotype of mature B cells from T21 and disomic (control) FL, FBM and CB samples were analysed by multiparameter flow cytometry. B cell receptor (BCR) repertoire was investigated by CDR3 length analysis using spectratyping. B cell development potential of T21 vs disomic $CD34^+$ cells was assessed in NOD/SCID/IL-$2Rg^{-/-}$ (NSG) xenograft assays. **Results.** The frequency of mature B cells ($CD34^-CD19^+$) was drastically reduced in T21 FL (n=8) compared to normal FL (n=5, 0.87±0. 27% vs. 7.66±2.9% respectively, p=0.03). Similar differences were observed in FBM (DS: n=3, 10.9±4.3% vs normal: n=3, 41.3±9.8%, p=0.049) and CB (DS: n=6, 4.6±2.1% vs normal: n=15, 14.3±2%, p=0.01). Despite the reduced B cell frequency, phenotypic maturation of B cells was similar in T21 and disomic samples. While nearly all B cells in normal and T21 fetal samples displayed a transitional phenotype ($CD34^-CD19^+CD10^+CD27^-$), CB samples contained mainly naïve ($CD34^-CD19^+CD10^-CD27^-$) cells with no significant difference in the proportion of naïve, transitional and memory cells between normal and T21 CB. Analysis of the BCR repertoire of $CD34^-CD19^+$ B cells by IgH spectratyping showed that fetal T21 mature B cells develop a fully diversified BCR repertoire comparable to that of gestation-matched disomic B cells consistent with a maturation block in DS at the Pro-B progenitor level prior to Ig gene rearrangement. To investigate whether the quantitative defect in B cell development is cell-intrinsic we transplanted 30,000 $CD34^+$ cells into NSG mice. Only 25% of T21 FL and none of the T21 FBM vs 100% of disomic samples showed BM engraftment after 12 weeks. Furthermore, while disomic FL $CD34^+$ cells gave rise predominantely to $CD34^-CD19^+$ BCs (77.8%±2.8% within the human $CD45^+$ population), engrafted T21 $CD34^+$ cells showed reduced ability to generate B cells (15.1% of the human $CD45^+$ cells) recapitulating the B cell ontogeny of human T21 fetal haemopoiesis consistent with the T21 fetal B cell differentiation defect being cell intrinsic. **Conclusions.** T21 and disomic mature fetal B cells display similar phenotypic patterns of differentiation and a fully diversified BCR repertoire. However, overall frequency of mature B cells in DS is severely reduced and results from a cell intrinsic process that leads to a maturation block at the Pro-B cell level.

0108

TRAIL IMPAIRS STEM CELL SURROGATES OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN PRECLINICAL TRANSPLANTATION ASSAYS

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Background. Cancer stem cells represent the most important target cells for anti-tumor therapy as they are uniquely capable to maintain tumor growth and to induce relapse. TRAIL (TNF-related apoptosis-inducing ligand) is a member of the TNF family which induces apoptosis in a wide variety of tumor cells while sparing normal cells. TRAIL is currently tested in phase I and II clinical trials, yet its ability to target cancer stem cells is currently unknown. **Aim.** Here we aimed to evaluate the effect of TRAIL on LICs using tumor cells derived from

children with precursor B-cell acute lymphoblastic leukemia (pre-B ALL). **Methods.** Primary ALL cells were passaged through NSG mice, freshly stimulated with TRAIL *in vitro* and subjected to limiting dilution transplantation assays *in vivo*. After 12-16 weeks, mice were evaluated for leukemic engraftment. **Results.** In all 3 pre-B ALL samples tested, *in vitro* treatment with TRAIL prior to transplantation of cells into mice significantly reduced their engraftment capability. TRAIL disabled leukemic engraftment by > 95 % in all 3 samples and increased the time to engraftment and completely disabled it. In a second, additive approach, TRAIL was used in a preclinical mouse model. Pre-B ALL samples were engrafted in NSG mice and leukemia-bearing mice were treated with TRAIL systemically at 7.5 mg / kg daily i.p. for 10 days. In this preclinical *in vivo* model, TRAIL treatment completely cured a proportion of animals harbouring patient-derived pre-B ALL xenografts. In mice developing ALL despite of TRAIL treatment, ALL cells required prolonged incubation periods upon secondary transplantations. **Summary and Conclusions.** Taken together, TRAIL significantly disabled the leukemia-initiating function of LICs from patient-derived pre-B ALL xenografts *in vitro* and eliminated LICs *in vivo*. Our data show that it is feasible, although technically demanding, to test apoptosis sensitivity of LICs. We conclude from these data that TRAIL constitutes an attractive future drug for treatment of ALL.

0109

MATRIX PROTEIN ?IGH3 FUNCTION IN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Background. Adult hematopoietic stem and progenitor cells (HSPC) reside in specific niches in the bone marrow (BM). Within this specialized microenvironment, the interactions of HSPC with adhesion molecules on neighbouring cells and extracellular matrix (ECM) components are thought to be crucial for the maintenance of the HSPC population.Comparative gene-expression profiling of purified HSPC in homeostatic and regenerative conditions identified the ECM protein βigh3 as being upregulated in regenerative BM. **Aims.** The aim of this project is to study the function of the ECM protein βigh3 inHSCself-renewal and differentiation. **Methods.** Cord-blood derived $CD34^+$ cells and/or bone-marrow derived mesenchymal stromal cells (MSC) were transduced by lentivirus to obtain overexpression or knock-down of βigh3 and these cells were analyzed in various colony-assays, differentiation-assays and (co)cultures. **Results.** High endogenous expression of βigh3 was observed in MSC, whereas expression in HSPC is low and increases as cells differentiate to monocytes.Overexpression of βigh3 in HSPC (on average 80-fold) accelerated megakaryopoiesis and increased the percentage of mature megakaryocytic cells from 16%±6% to 30%±7 (n=4). In contrast, overexpression of βigh3 in HSPC reduced granulocytic proliferation (80%±11 reduction at day 7, n=3) and decreased the number of early colony-forming-unit-granulocyte-monocyte (CFU-GM) progenitors (21%±7 reduction, n=7), while the erythrocytic differentiation was not affected. Together these data indicate that overexpression of βigh3 differentially affects distinct hematopoietic lineages. Overexpression of βigh3 resulted in a progressive reduction over time of the quality of HSPC, determined as the long-term-culture-colony-initiating-cells (LTC-CFC) formation after co-culture (in week 2, 4 and 6 respectively a reduction of 23%, 61% and 92%), indicating again that βigh3 drives differentiation of HSPC.Knock-down of βigh3 in HSPC resulted in a more than 2-fold reduction of CFU-GM formation and an even more pronounced decrease (3.3-fold) in the number of colony-forming-unit-erythrocyte, which is explained by reduced proliferation of βigh3 knock-down cells. Accordingly, knock-down of βigh3 resulted in reduced cell numbers in MSC as well as HSPC cultures (at day 13 from 20- to 6-fold, per input cell), in addition to a reduction in the percentage of cells in cell cycle progression and reduced expression of cell cycle genes. Together these data indicate that βigh3 plays a role in cell proliferation.Interestingly, knock-down of βigh3 in HSPC increased the potential of HSPC to form cobblestone-areas under a layer of stromal feeder cells. The increased number of cobblestone-area-forming-cells (CAFC) detected at week 4 (1.7-fold) and 6 (1.8-fold) in this assay, and the increase in LTC-CFC formation (15.5-fold) indicated that reduction of βigh3 levels maintains renewal capacity of HSPC.**Summary and Conclusions.** βigh3 affects lineage commitment of HSPC and is an important factor in HSPC proliferation. Overexpression of βigh3 in HSPC accelerates megakaryocytic differentiation, while decreasing granulocytic proliferation and CFU-GM formation, suggesting that βigh3 drives lineage commitment of HSPC. Knock-down of βigh3 in HSPC stimulates CAFC formation, indicating that decreased βigh3 levels maintain undifferentiated HSPC. We postulate that the levels of βigh3 have to be tightly regulated in the (regenerative) bone marrow microenvironment to balance the self-renewal and differentiation of HSPC.

0110

PROTECTION OF HEMATOPOIETIC STEM CELLS (HSC) AND PROGENITORS AGAINST OXYDATIVE STRESS BY CXCR4/SDF-1 AXIS

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Background. Reactive Oxygen species (ROS) are a heterogeneous group of molecules and free radicals derived from diatomic oxygen. Many physiologic and pathological states such as aging, infections or cancer are accompanied by excessive cellular ROS production leading to a state known as oxidative stress. The chemokine receptor CXCR4 and its ligand Stromal cell Derived Factor 1 (SDF-1) have been shown to provide a key role in the control of HSC and hematopoietic progenitor biology. We have recently shown that disruption of the CXCR4 receptor induces progressive loss of HSC and progenitors in the peripheral blood, leading to a sharp decline in CXCR4-/- hematopoiesis over time. **Aims.** We hypothesized that exhaustion of the bone marrow (BM) stem cell pool in CXCR4 -/- mice is due to increased oxidative stress in HSC and progenitors and that CXCR4/SDF-1 confers stress resistance to HSC. **Methods.** We induced *in vitro* oxidative stress in mature and primitive c-kit+Sca1+ lineage low/- (LSK) hematopoietic cells with the oxydant L-buthionine sulfoximine (BSO). The effect of SDF-1 on BSO-induced ROS level increase was evaluated with 2',7'- dichlorofluorescein (DCF), a molecular probe for general ROS detection. The capacity of BM cells or purified BM subpopulations to give rise to hematopoietic progenitors was quantified by seeding treated and untreated cells on semi-solid medium. DNA double-strand breaks induced by BSO were evaluated in the absence and presence of SDF-1 by immunofluorescence microscopy using an antibody specific for phospho-histone gamma H2AX. To evaluate CXCR4 functions on oxidative stress *in vivo*, CXCR4 chimeric mice were generated by transplanting embryonic day 14.5 CXCR4+/+ and CXCR4-/- foetal liver cells to lethally irradiated mice. Stably reconstituted mice were supplemented or not with N-acetylcysteine (NAC), a known ROS scavenger (H_2O_2), in the drinking water for one month. Cell cycle status, apoptosis, ROS level, DNA double-strand breaks and hematopoietic progenitors as well as phenotypically defined HSC-SLAM (LSK CD150+CD48-) numbers were quantified in BM and blood. **Results.** : i) Basal ROS production is heterogeneous in hematopoietic cells, LSK and LSK-SLAM cells presenting very low ROS levels compared to more mature cells. ii) SDF-1 or NAC addition to the culture medium prevents BSO effects on ROS augmentation and rescues BSO-induced decrease in hematopoietic clonogenic function. BSO-induced DNA damage is rescued by SDF-1 at the HSC level. iii) CXCR4-/- chimeric mice treated with NAC exhibited lower HSC ROS levels and higher numbers of medullary HSC, indicating that NAC treatment prevents exhaustion of the medullary stem cell pool. In the BM, CXCR4-/- SLAM cells are hyper proliferative in both, NAC treated and untreated mice, suggesting that the rescue effect of NAC is independent from cell cycle status. In contrast, NAC treatment results in reduced apoptosis and DNA damage in primitive cells. **Conclusions.** CXCR4/SDF-axis signalling is essential for protection of hematopoietic progenitors and HSCs against acute and chronic oxidative stress.

0111

THE EXTRACELLULAR MATRIX PROTEIN SLIT3 PROMOTES ERYTHROPOIESIS

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Background. Slit extracellular matrix proteins are expressed by bone marrow (BM) stromal cells, whereas their receptors, the Roundabout (Robo) proteins, are expressed by hematopoietic stem- and progenitor cells (HSPC). We previously showed that homing of HSPCs to the BM was enhanced when the HSPCs were pre-treated with Slit3. **Aims.** The aim of this study was to determine whether and by what molecular mechanism Slit3 affects the proliferation and differentiation of HSPCs. **Methods.** The role of Slit3 was examined at distinct levels in the hematopoietic hierarchy. To investigate the role of Slit3 at the level of various stem and progenitor cell subsets, a colony-formation-unit-in culture assay was performed. To this end, cord blood (CB)-derived HSPCs and CB and BM-derived HSPCs subsets, i.e. hematopoietic stem cells (HSCs), common myeloid progenitors (CMPs), megakaryocyte-erythrocyte progenitors (MEPs) and granulocyte-monocyte progenitors (GMPs), were plated in semi-solid medium supplemented with growth factors in the presence or absence of Slit3. Next, to examine whether Slit3 affects the differentiation of HSPCs, the HSPCs were cultured in the presence or absence of Slit3 in serum-free liquid medium containing TPO, SCF and Flt3. We also investigated the role of Slit3

during differentiation of HSPCs towards erythroblasts in the presence of EPO, SCF and IL3. To unravel the cellular signaling induced by Slit3, we transfected HEK cells with Robo1 and Nck2, performed immunoprecipitation to identify interacting proteins and analyzed the phosphorylation status of all proteins in the complex. **Results.** Slit3 did not change the number of colony-forming units in the granulocyte-monocyte lineage or the megakaryocytic lineage, but increased the number of the burst-forming unit erythroid (BFU-E) progenitors by 2.7-fold. BFU-E colony formation from the HSC, the CMP and the MEP population increased in the presence of Slit3, whereas BFU-E colonies did not grow out from the GMP fraction as expected. During liquid culture of HSPC, Slit3 slightly increased the total number of nucleated cells and the number of cells with an immature erythroblast phenotype ($CD34^-CD45^-CD36^+CD71^+$) at day 11 by 1.8-fold. In the absence of EPO, the cells did not differentiate to more mature glycophorin A/$CD235^+$ erythroblasts. Notably, the frequency of BFU-E increased after 4, 7 and 11 days of culture with a maximal increase of 4.2-fold at day 7. During erythroblast differentiation culture, the number of $CD235^+$ cells was increased in the presence of Slit3 at day 7 and day 11 of culture, with a maximum of 2.3-fold at day 11. Concerning the molecular mechanism, we observed that Robo1 interacts with the adaptor proteins Nck and p130Cas. Slit3 decreased the tyrosine phosphorylation of Robo1 and increased the tyrosine phosphorylation of p130Cas, resulting in recruitment of the tyrosine kinase Lyn to the Robo1-Nck-p130Cas complex. Interestingly, this member of the Src family of tyrosine kinases is implicated in the regulation of erythropoiesis. **Conclusions.** Together, our data indicate that Slit3 promotes expansion of the erythroid progenitor compartment which results in an increased output of erythroid cells. Downstream of Slit3 this may involve activation of the Lyn kinase.

0112

CENTRAL MEMORY CD8 T CELLS REGULATE HEMATOPOIETIC STEM CELL FUNCTION

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After immune activation, effector T cells, including virus-specific CD8 T cells, are known to migrate to the bone marrow (BM); however their function at this site is unknown. Since depletion of T cells from allogenic BM grafts compromisesHSCengraftment, we hypothesize that T cells can directly influence the balance between differentiation and self renewal of hematopoietic stem cells (HSCs). To test the ability of T cells to affect hematopoiesis, we performed liquid co-cultures of HSCs and T cells isolated from mouse BM. Here we show that T cells localized in the BM are able to enhanceHSCdifferentiation as well as their self-renewal capacity. This feature is specific for BM central memory (CM) CD8 T cells, since other T cell subsets are not able to affect HSCs to the same extent. Moreover, depletion of CM CD8 T cells from the total BM T cell pool abrogates the impact onHSCdifferentiation and self-renewal. In vitro studies showed that BM CM CD8 T cells do not affect quiescence ofHSCs, but do enhance their proliferative capacity. We also show that supernatant from CM CD8 T cells is sufficient to increaseHSCsnumbers *in vitro*. Interestingly, competitive transplantation assays showed thatHSCscultured with CM CD8 T cells-derived supernatant contribute much better to leukocyte formation than medium-treated HSCs. This effect is seen in both the myeloid and lymphoid compartment, indicating that CM CD8 T cells are able to release soluble factors that support and enhance the multilineage reconstitution capacity of HSCs. Functional studies with blocking antibodies or knock-out mice showed that the supernatant-mediated effect is not caused by the hematopoietic cytokines IL3, IL6, IL21, GM-CSF, TNF-a or IFNg. Preliminary data indicate that this feedback mechanism of the immune system on the hematopoietic process in the bone marrow is also present in the human situation, since autologous BM T cells increase the numbers of human HSCs, as well as their differentiation capacity.Overall, these findings demonstrate that T cells have an important function in the BM and that CM CD8 T cells can directly influenceHSChomeostasis. We postulate that virus-specific CD8 T cells migrate to the BM after infection in order to enhance the function of HSCs in their ability to replenish theHSC/progenitor cell compartment and restore blood cell numbers.

0113

G PROTEIN-COUPLED RECEPTOR CROSSTALK IN HEMATOPOIETIC STEM AND PROGENITOR CELLS: ROLE OF HOMOLOGOUS AND HETEROLOGOUS DESENSITIZATION

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Background. Hematopoietic stem and progenitor cells (HPC) express a variety of G protein-coupled receptors (GPCR), which all could play important roles in HPC trafficking. In other cell types, a complex network of crosstalk between different GPCR has been described. In HPC however, mechanisms regulating differential expression and function of GPCR are largely unknown. **Aims.** We analyzed crosstalk of CXCR4 and CysLT1, the two GPCR with the strongest cellular responses, in mobilized CD34+ HPC, using calcium signaling and actin polymerization as a functional read-out, and characterized the underlying signal transduction pathways. **Methods.** Human mobilized CD34+ HPC were isolated from the peripheral blood. Calcium fluxes and actin polymerization in response to receptor activation were measured using a fluorescent calcium indicator and phalloidin-FITC. Phosphorylation of kinases involved in signal transduction was assessed by Western blot. **Results.** Activation of CXCR4 as well as CysLT1 resulted in homologous desensitization as measured by absence of calcium fluxes and actin polymerization after repeated stimulation. Regarding calcium fluxes, heterologous cross-desensitization of CXCR4 by preceeding activation of CysLT1 and vice versa of CysLT1 by activation of CXCR4 was not observed. In contrast, heterologous desensitization of CysLT1-mediated actin polymerization was induced by preceeding activation of CXCR4, but not vice versa. This observation correlated with the inhibitory effect of protein kinase C (PKC) blocking with BisI and PKCzeta pseudosubstrate, which was observed analyzing actin polymerization in response only to CXCR4, but not to CysLT1 activation. Calcium fluxes mediated by either CXCR4 or CysLT1 could not be blocked with BisI and PKCzeta pseudosubstrate. Interestingly, Pyk2 phosphorylation was abrogated by pertussis toxin (Gi protein inhibitor) only after activation of CXCR4, but not CysLT1, revealing also a differential involvement of G proteins. **Conclusions.** In CD34+ HPC, homologous desensitization, which is required for rapid termination of receptor activation, occurs after activation of CXCR4 and CysLT1, and is due to receptor internalization, as both calcium fluxes and actin polymerization are inhibited. Heterologous desensitization of CysLT1 after preceeding activation of CXCR4 is restricted to actin polymerization and mediated by PKC signaling rather than receptor internalization. As signaling of CXCR4 is not influenced by preceeding CysLT1 activation, and actin polymerization represents a prerequisite for cell migration, a hierarchy exists in the control of HPC migration by different GPCR, with a dominant role for CXCR4. These findings confirm the particular importance of CXCR4 in the regulation of stem cell trafficking.

0114

IRRADIATION INDUCED GROWTH FACTOR FOR HEMATOPOIETIC MICROENVIRONMENT

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Background. Mesenchymal stem cells (MSCs) are capable to transfer hematopoietic microenvironment. After implantation of femur bone marrow plug under the renal capsule of syngeneic recipients the ectopic hematopoietic foci are formed. Stromal cells in such foci are derived from donor MSCs while hematopoietic cells have the recipient's origin. The size of the foci formed (estimated by nucleated cell number) is proportional to the femur equivalent transplanted. It is known that the foci are enlarged 2-3 times in (sub)lethally irradiated recipients. It was shown that soluble factor that stimulates the growth of stromal microenvironment of the foci is produced in and secreted into the blood serum by irradiated bones.The aim of the study was to determine the nature of this stromal growth factor. **Methods.** The expression level of 75 genes associated with MSCs was analyzed in murine normal and irradiated bones by RQ-PCR. The expression levels of only 2 genes coding soluble secreted factors-IL-1beta and VEGF were elevated in irradiated bones (134 and 4 times, respectively). To verify the ability of IL-1beta to increase the size of ectopic foci it was administered to syngeneic (C57Bl x CBA) F1 recipients of bone marrow plugs implanted under their renal capsules. IL-1beta have been injected in transplanted mice intraperitoneally and subcutaneously in doses 25, 100, 200 and 400 pg per mouse once a day for the first 3 weeks after implantation of the bone marrow plug. Dose was guided by the concentration of IL-1beta measured by ELISA in the serum of irradiated mice. The size of each focus was determined 6 weeks after implantation by nucleated cells count and by weighting the bone shells.Results. The size of ectopic hematopoietic foci in control group was 6.5 $\pm 0.5 \cdot 10^6$ cells while it was larger in every injected groups - 7.2±0.8, 10.8± 0.5,

10.6, and 7.7±1.2·10^6 of nucleated cells for 25, 100, 200 and 400 pg of IL-1beta, respectively. Thus the effect was dose-dependent with the optimal dose of IL-1beta for stimulating hematopoietic microenvironment being 100 pg. The weight of bone shells was 2.8±0.1 mg for control group, 3.7±0.1, 4.8±0.1, 3.5 and 2.3 ±0.3 mg for each experimental group, respectively. These results demonstrate that injections of 100 pg of IL-1beta lead to the formation of larger foci of hematopoiesis with both higher number of nucleated cells and larger bone shells. **Conclusions.** Here we demonstrate, for the first time, the ability of IL-1beta to stimulate the development of hematopoietic microenvironment *in vivo*. IL-1beta could be not the single protein to induce the growth of stromal precursor cells. Another potential stromal growth factor *in vivo* could be VEGF, moreover IL-1beta might stimulate the production of some other factors capable of promoting the growth of hematopoietic microenvironment. The investigation of the mechanisms of IL-1beta action may help in determining the hierarchy of MSCs. Such novel stromal growth factor may be useful for treatment of several diseases affecting either hematopoietic microenvironment or bone defects.

0115

EXPRESSION OF PSTAT5 IN CD34+/CD38- STEM CELLS IN JAK2 V617F+ MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Signal transducer and activator of transcription 5 (STAT5) has recently been implicated as a major regulator of growth and survival of neoplastic cells in various myeloid malignancies. In essential thrombocytosis (ET), polycythemia vera (PV) and primary myelofibrosis (PMF), the JAK2 V617F mutant is often detectable, and is considered to lead to activation of STAT5 in the cytoplasm of neoplastic cells. We examined the expression and function of STAT5 in primary neoplastic cells obtained from patients with ET, PV, and PMF, and in the JAK2 V617F+ erythroid leukemia cell line HEL. As assessed by immunocytochemistry, primary neoplastic JAK2 V617F+ cells and HEL cells expressed phosphorylated (p) STAT5 in their cytoplasm as well as in their nuclei. Western blot analysis confirmed that both the cytoplasmic as well as the nuclear extracts of HEL contain pSTAT5. We next examined pSTAT5 expression in CD34+/CD38- stem cells in 9 patients with JAK2 V617F+ myeloproliferative neoplasms (MPN) by flow cytometry. In all patients tested, the CD34+/CD38- stem cells were found to express pSTAT5, whereas CD34+/CD38- stem cells in the normal/reactive bone marrow did not express pSTAT5. In a next step, we applied the STAT5-targeting drugs piceatannol and pimozide. As assessed by ^{3}H-thymidine uptake, both drugs were found to inhibit the proliferation of HEL cells and of primary neoplastic cells in a dose-dependent manner. Both drugs were also found to induce apoptosis in HEL cells as determined by active caspase 3 staining. In summary, our data show that neoplastic cells in JAK2 V617F+ neoplasms express pSTAT5 in their cytoplasm and in their nuclei, and that targeting of STAT5 may be a novel interesting approach to control the expansion of neoplastic cells in MPN. Since pSTAT5 is also expressed in stem cell-enriched (CD34+/CD38-) fractions of neoplastic cells, STAT5 may serve as a promising new target in JAK2-mutated MPN.

Granulocytes

0116

CONGENITAL AND CYCLIC NEUTROPENIA WITH ELANE MUTATIONS: GENOTYPE-PHAENOTYPE CORRELATION

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Background. Mutations in the neutrophil elastase gene (*ELANE*) can be detected in more than 80% of cyclic neutropenia patients (CyN) and in the majority of patients suffering from autosomal dominant or sporadic congenital neutropenia (CN). **Aims and Methods.** Based on the data of the European Branch of the Severe Chronic Neutropenia Registry (SCNIR), we analysed genotype-phenotype correlation in ELANE-CN and ELANE-CyN - in particular with regard to the course of blood counts, bone marrow results, infections, response to therapy, risk of leukaemia and long term prognosis. **Results.** To-date, the SCNIR has collected data on 332 CN and 69 CyN patients from 23 European countries. Molecular genetic testing was performed in 125 of 332 and 32 of 69 CyN patients. In 83 out of 125 tested CN patients and in 26 out of 32 tested patients *ELANE* mutations were found. *ELANE* mutations are spread over all parts of the gene in both, CN and CyN, subtypes. So far, a number of mutations are present in CN or CyN only, but there are also shared mutations for both clinical subtypes. Therefore, the identified *ELANE* mutation does not allow discrimination between CN and CyN. Diagnosis is still depending on the clinical course of neutrophils. Differences between ELANE-CN and ELANE-CyN can be found in response to G-CSF treatment, bone marrow morphology, type of infections as well as risk of leukemic transformation. To-date, approximately 11% of the CN and none of the CyN patients developed leukaemia. In general, patients requiring higher doses of G-CSF are at greater risk.Intriguingly, in two patients in addition to *ELANE* we detected homozygous mutations in *G6PC3* and *HAX1*, respectively (digenic mutations).Due to the good treatment response to G-CSF, life expectancy is now prolonged: 41 of the 83 ELANE-CN and 15 out of the 26 ELANE-CyN patients have reached adulthood so far. A first survey on parenthood revealed that nine ELANE-CN (5 men, 4 women) and four ELANE CyN (2 men, 2 women) patients have children. Thirteen out of these 21 children suffer from neutropenia too (10/13 of ELANE-CN parents and 3/8 of ELANE-CyN parents). **Conclusions**. Based on the detected *ELANE* mutation it is not possible to assign to ELANE-CN or ELANE-CyN or determine the patient's risk for leukemic transformation.

0117

ACETYLATION OF MYELOID-SPECIFIC TRANSCRIPTION FACTOR C/EBP ALPHA

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Previously, we described new mechanism of G-CSF-triggered granulocytic differentiation via activation of the enzyme Nicotinamide Phosphorybosyltransferase (Nampt) leading to NAD$^+$ production and activation of NAD$^+$-dependent protein deacetylases SIRT1 and SIRT2.In order to investigate the mechanism of Nampt-triggered myeloid differentiation, we investigated whether myeloid-specific transcription factor C/EBPα could be acetylated. We found that SIRT1 and SIRT2 bind to and activate C/EBPα. We found that C/EBPa is acetylated on Lys 161 and generated rabbit polyclonal antibody specifically recognised acetyl-Lys 161 C/EBPa. We further analysed intracellular localization of acetylated C/EBPa and found that in acute myeloid leukemia cell lines NB4 and HL60 as well as in primary hematopoietic CD34$^+$ cells acetylated C/EBPa was localized in the nucleus. G-CSF treatment of CD34$^+$ cells or ATRA treatment of NB4 cells resulted in the deacetylation of C/EBPa. To evaluate the involvement of Nampt in the deacetylation of C/EBPa we treated NB4 and HL60 cells with the specific inhibitor of Nampt, FK866 and found dramatically elevated levels of acetylated C/EBPa after inhibition of Nampt. Similarly, inhibition of SIRT2 using specific inhibitor AC93253 also resulted in elevated levels of acetylated C/EBPa. Moreover, Nampt and SIRT1 significantly enhanced C/EBPa-triggered activation of reporter gene constructs of C/EBPa target genes G-CSF and G-CSFR, and by this induced granulocytic differentiation. Taken together, Nampt/SIRT dependent deacetylation of C/EBPa is involved in granulopoiesis.

0118

SECRETORY LEUKOCYTE PROTEASE INHIBITOR (SLPI) PLAYS AN IMPORTANT ROLE IN MYELOID DIFFERENTIATION

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Secretory Leukocyte Protease Inhibitor (SLPI) is a cationic serine protease inhibitor with antiprotease, primarily anti-Neutrophil Elastase (NE), activities. Moreover, SLPI modulates intracellular signal transduction pathways such as NF-kB and Erk. The molecular interaction and the balance between NE and SLPI is tightly regulated. On the one side, NE upregulates the SLPI expression and at the other hand SLPI inhibits the NE-induced degradation of proteins.In severe congenital neutropenia (CN) patients with ELA2 mutations, mutated NE protein may activate unfolded protein response (UPR) and by this induce apoptosis and diminished differentiation of hematopoietic cells. In the present study we aimed to measure the amount of NE inhibitor, SLPI in myeloid cells and in plasma of CN patients.We identified severe diminished levels of SLPI mRNA in $CD33^+$ myeloid cells and in PMNs of CN patients, as compared to patients with cyclic neutropenia (CyN) and to healthy individuals. SLPI protein levels in plasma of CN patients were also significantly reduced. We further analysed whether diminished levels of SLPI are associated with the "maturation arrest" of myeloid cells seen in CN patients. We inhibited SLPI in the myeloid cell line NB4 and in hematopoietic $CD34^+$ cells using lentivirus-based transduction with SLPI-specific shRNA and analysed ATRA- or G-CSF-triggered myeloid differentiation, respectively. Indeed, myeloid differentiation was diminished in NB4 cells and in $CD34^+$ cells transduced with SLPI-specific shRNA, as compared to control shRNA transduced cells, which was accompanied by G_0/G_1 cell cycle arrest of SLPI shRNA transduced cells.We next analysed the mechanisms of diminished myeloid differentiation after inhibition of SLPI. SLPI is known to be involved in phosphorylation of Erk1/2 protein. Erk1/2 protein is involved in myeloid differentiation. We found that ATRA treatment of NB4 cells transduced with ctrl shRNA resulted in phosphorylation of Erk1/2 with a maximum on day 2 of treatment. Interestingly, we measured reduced Erk1/2 phosphorylation in ATRA-treated NB4 cells transduced with SLPI shRNA. Moreover, it has been shown that Erk1/2 phosphorylates LEF-1 transcription factor, essential for granulocytic differentiation and we found diminished levels of phosphorylated LEF-1 in SLPI shRNA transduced cells. Previously, we demonstrated severe diminished LEF-1 expression levels in myeloid cells of CN patients. Therefore, we concluded that SLPI is involved in myeloid differentiation by regulation of Erk1/2 dependent LEF-1 expression.

0119

A ROLE OF GADD45B PROTEIN IN MYELOID DIFFERENTIATION AND STRESS RESPONSE OF HUMAN HEMATOPOIETIC CELLS

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Growth arrest and DNA-damage-inducible, beta (GADD45b) protein modulates stress responses in mouse myeloid cells. Recently, we demonstrated, that in patients with severe congenital neutropenia (CN) daily treatment with high doses of G-CSF stimulated C/EBPß-dependent emergency stress-induced granulopoiesis.Based on these findings, we assumed that GADD45b could play a role in the induction of granulopoiesis in CN patients. We assessed GADD45b expression levels in "arrested"promyelocytes of CN patients and surprisingly found severe downregulation of GADD45b expression in CN promyelocytes, in comparison to cyclic neutropenia patients and to G-CSF-treated healthy individuals. Expression of GADD45b target genes (e.g. cyclin B1, p27, CDK6, CDK4, bcl-xl) was also significantly reduced. We further evaluated a possible involvement of GADD45b in myeloid differentiation and stress response of human myeloid cells. GADD45b protein is known to be activated and to migrate into the nucleus after induction of DNA damage (e.g. ultraviolet (UV) irradiation). We found that UV irradiation of the NB4 but not THP1 acute myeloid leukemia cell line induces nuclear GADD45b. Therefore, we further analysed functions of GADD45b in myeloid differentiation and DNA damage in NB4 cells. We found that GADD45b was activated and migrated into the nucleus upon treatment of NB4 cells with ATRA. Inhibition of GADD45b in these cells led to dramatically reduced ATRA-triggered myeloid differentiation, which was in line with diminished expression of myeloid-specific transcription factor C/EBPß, which triggers "stress-induced" myeloid differentiation, but also of C/EBPa, responsible for "steady-state" granulopoiesis. Moreover, transduction of NB4 cells with GADD45b shRNA resulted in significantly enhanced sensitivity to UV irradiation, as documented by elevated apoptosis and reduced proliferation. This was in line with diminished mRNA expression of anti-apoptotic genes bcl-2, mcl-1, survivin as well as elevated mRNA expression of pro-apoptotic gene bad in GADD45b shRNA transduced cells, as compared to ctrl shRNA transduced cells. p21 mRNA expression was also significantly downregulated after inhibition of GADD45b mRNA.Taken together, GADD45b plays an important role in myeloid differentiation and stress response of acute myeloid leukemia cell line NB4. Studies of the effects of GADD45b in myeloid differentiation of primary hematopoietic cells in healthy individuals and in CN patients are ongoing.

0120

NAMPT-DEPENDENT DEACETYLATION OF THE HEMATOPOIETIC-SPECIFIC LYN-SUBSTRATE 1 (HCLS1)

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Recently, we demonstrated an essential role of the hematopoietic cell-specific Lyn substrate 1 (HCLS1 or HS1) protein in myeloid differentiation of human and mouse hematopoietic cells. HCLS1 is an interaction partner of HAX1, which is mutated in patients with severe congenital neutropenia (CN). In these patients HCLS1 expression and functions are severely downregulated, leading to "maturation arrest"of myelopoiesis. We also described the new pathway of myeloid differentiation downstream of G-CSF in healthy individuals and in CN patients: G-CSF induced Nampt and NAD^+, which activated NAD^+-dependent protein deacetylases, sirtuins. A huge amount of proteins are post-translationally modified by acetylation or deacetylation. However, it is unclear whether changes in the acetylation status activates or suppresses functions of proteins.In the present study we analyzed if HCLS1 protein could be de-/acetylated and if de-/acetylation of HCLS1 affects its functions during myeloid differentiation. We found that HCLS1 is acetylated on three lysines in the acute myeloid leukemia cell lines NB4 and HL60 as well as in $CD34^+$ hematopoietic cells. We also found that SIRT1 and SIRT2 interacted with HCLS1 protein in NB4 cells. Moreover, specific inhibition of Nampt (FK866) in NB4 cells led to enhanced acetylation of HCLS1 on Lys 123 and 241. Inhibition of SIRT2 (AC93253) resulted in elevated HCLS1 acetylation on Lys 123 and 192. Moreover, treatment of $CD34^+$ cells with G-CSF induced interaction between SIRT2 and HCLS. Effects of Nampt/SIRT-triggered deacetylation on HCLS1 functions remain to be investigated.

0121

DETERMINING THE FREQUENCY IN HEALTHY INDIVIDUALS OF PERIPHERAL BLOOD GRANULOCYTES CARRYING ACQUIRED SOMATIC MUTATIONS OF THE X-LINKED GENE PIGA

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Background. The force that drives the transformation of a normal cell into a neoplastic cell is the accumulation of a discrete set of somatic mutations. The occurrence of mutations is an inherent risk associated with DNA replication and cell division. Estimates of the human somatic mutation rate (μ) have been previously obtained from studies on dermal fibroblasts and on lymphoblastoid cells: however, the methodology required has been laborious and did not lend itself to the analysis of large numbers of samples. We have previously shown that the X-linked gene *PIG-A*, since its protein product is required for numerous glycosyl-phosphatidylinositol-anchored proteins to become surface bound, is a good sentinel gene for studying the frequency of spontaneous somatic mutations. **Aims.** To establish that a parameter related to μ, namely the frequency *f* of mutant cells, can be reliably and reproducibly measured on peripheral blood samples by flow cytometry analysis of granulocytes. **Methods.** 5 millions granulocytes, separated by a double density gradient, were stained with a mixture of 3 PE-moAbs that recognize 3 different GPI-linked proteins (CD59, CD55, CD24), plus one pan-leukocyte marker (anti CD45-APC) and one granulocyte marker (anti-CD11b-FITC). Granulocytes that are deficient in GPI-anchored proteins because of a *PIG-A* gene mutation are counted by FACS analysis. Mutant frequency, *f*, was calculated as the number of GPI-negative events per million cells. **Results.** We found that two independent measurement of *f* on the same sample (split samples) were very similar, with a coefficient of variation (CV) of 44.7%. From 32 volunteers we have collected 3 repeat samples with time intervals from 2 to 14 weeks (repeat samples): the CV of *f* in these repeat samples was 44.3%. Finally, we have studied *f* as a biological variate in 142 normal subjects. We have found that in this normal human popula-

tion Log *f* has a normal distribution. The variability of *f* spans a 80-fold range, from less than 1 to 37.5x10^{-6}, with a median value of 4.9x10^{-6}, and with 80% of values below 10.2x10^{-6}. **Conclusions.** We have shown that we can accurately measure, from a small sample of peripheral blood, the proportion of *PIG-A* mutant granulocytes (*f*). Since split samples and repeat samples have an almost identical CV (44.7 vs. 44.3%), we can infer that *f* is a relatively stable individual characteristic. The normal distribution of Log *f* is reminiscent of that of fundamental quantitative traits, such as height and many others. The methodology we describe will lend itself to investigating genetic factors that underlie the variation in the somatic mutation rate, as well as environmental factors that may affect it, including chemotherapy. It will be also possible to test whether *f* correlates with the risk of cancer.

0122

EFFICACY AND SAFETY OF BALUGRASTIM COMPARED WITH PEGFILGRASTIM IN PATIENTS WITH BREAST CANCER WHO ARE RECEIVING CHEMOTHERAPY

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Background. Patients receiving cancer chemotherapy are at an increased risk of neutropenia. Recombinant granulocyte colony stimulating factors (G-CSFs) have been developed to stimulate proliferation and differentiation of neutrophils. Pegfilgrastim is a pegylated recombinant G-CSF that allows for once-per-cycle dosing. Balugrastim is a long-acting G-CSF composed of a genetic fusion between recombinant human serum albumin and G-CSF. **Aims.** The objective of this study was to compare the efficacy and safety of balugrastim and pegfilgrastim in patients with histologically or cytologically confirmed breast cancer who were scheduled to receive doxorubicin and docetaxel. **Methods.** In this double-blind, randomized, active-comparator, noninferiority trial, patients with ≥1.5x10^9 neutrophils/L, and ≥100x10^9 platelets/L were randomly assigned to subcutaneous injections of balugrastim 40 mg (n=153) or pegfilgrastim 6 mg (n=151) with stratifications for weight, prior chemotherapy exposure, and global location. The primary efficacy endpoint was the duration of severe neutropenia (days with an absolute neutrophil count <0.5x10^9 cells/L) during cycle 1 for the population of patients who did not have major protocol violations. **Results.** Mean duration of severe neutropenia in cycle 1 was 1.1 days in the balugrastim group and 1.0 days in the pegfilgrastim group (95% CI for difference between groups -0.13 to 0.37). Fifty-eight percent of patients in the balugrastim group and 59% in the pegfilgrastim group had severe neutropenia during cycle 1 (95% CI for difference between groups -11.98% to 10.41%). Two and 4 patients, respectively, had febrile neutropenia during cycle 1; no patients in either group had febrile neutropenia during cycles 2-4. Twenty percent of patients in the balugrastim group and 19% in the pegfilgrastim group had adverse events that the investigator considered to be related to study medication. Six and 7 patients, respectively, had serious adverse events. **Conclusions.** The results of this study support the noninferiority of balugrastim versus pegfilgrastim, demonstrating that both compounds have comparable efficacy. There were no unexpected safety events.

0123

A SEMI-QUANTITATIVE MAGNETIC RESONANCE IMAGING METHOD FOR ASSESSING SKELETAL INVOLVEMENT IN NON-NEURONOPATHIC GAUCHER DISEASE

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Background. Gaucher disease (GD) is a lysosomal storage disease characterized by a genetic disruption in the metabolic breakdown of glucocerebroside caused by the lack of the enzyme beta-glucocerebrosidase. Bone complications are common in the non-neuronopathic form of GD and lead to considerable pain, limitations in mobility and negative impact on the quality of life. Skeletal manifestations include bone marrow infiltration, bone loss, lytic lesions and osteonecrosis. Magnetic resonance imaging (MRI) is the most sensitive procedure for assessing skeletal involvement in patients with GD and semi quantitative MRI is a very useful procedure for evaluating the bone marrow burden. **Aims.** The aim of this study was to develop a semi quantitative scoring system, based on MRI, to evaluate the bone marrow involvement in non-neuronopathic GD. **Methods.** We studied 27 adult patients (15M/12F, median age 44.5 years, range: 18-71 years) with the non-neuronopathic type of GD. MRI of the femoral bones was performed in all patients. We implemented T1-weighted spin echo (T1W) sequences, proton density sequences with fat suppression (PDFS) to further evaluate the detected changes on T1W sequence and short time inversion recovery (STIR) sequences for the evaluation of disease activity. We also performed a thorough comparison of T1W and PDFS images targeting in the detection of the remodeling process of the affected bone. In all patients we performed evaluation of bone infiltration on T1W images in affected areas as opposed with those of healthy regions and in conjunction with measurements taken from subcutaneous fat of both thighs of the same patients. We also studied 27 healthy individuals who had MRI scans for various other reasons, i.e. undiagnosed femur pain to exclude arthritis. **Results.** The MRI findings were independently evaluated by two experienced MR imaging specialists. The normal rate of subcutaneous fat of both healthy and affected individuals was fluctuated from 210-400 pixels and those of bony fat tissue from 120-180 pixels. On the contrary the affected values were found to vary from 15-80 pixels. In order to reduce the possibility of false positive and false negative values we combined all the region of interest (ROI) measurements with the degree of infiltration detected on T1W images. At the end we combined the above MRI findings and introduced the following classification: stage I: ROI 1/2 of normal values and bone infiltration up to 30%; stage II: ROI 1/3 of normal values and bone infiltration from 30 to 60%; stage III: ROI 1/4 of normal values and bone infiltration from 60% to 80% and stage IV: detection of epiphyseal infiltration, osteonecrosis and deformity regardless of the ROI'S values. All but one patient had abnormal MRI findings: 3 (11%) had stage I MRI abnormalities, 18 (66%) had stage II, 3 (11%) stage III and 2 (7%) stage IV. **Summary and Conclusions.** Our study suggests that the above semi quantitative MRI method is relatively easy to be performed compared to all other available methods for the non-neuronopathic GD. Furthermore, this sensitive MR technique would be useful for the evaluation of bone marrow involvement in this GD type.

Chronic lymphocytic leukemia - Biology 1

0124

MONOCYTIC POPULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA SHOWS ALTERED COMPOSITION AND PROANGIOGENIC, IMMUNOSUPPRESSIVE AND INVASIVE PROPERTIES

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Background. Macrophages are highly versatile cells and are prominent in the stromal compartment of virtually all types of malignancies. When migrating into tumor sites, they are exposed to stimuli of tumor cells that "educate"them to acquire a distinct phenotype of immunosuppression, angiogenesis and invasiveness. Macrophages reside in tissues infiltrated by chronic lymphocytic leukemia (CLL) B-cells and the extent of infiltration is associated with adverse prognostic factors. Moreover, nurse-like cells (NLCs), that differentiate from CD14+ monocytes into large/adherent cells, protect CLL from undergoing spontaneous or drug-induced apoptosis. **Aims.** We investigated whether CLL cells may influence the phenotype of monocytic population to promote a protective, pro-angiogenic and invasive profile. **Methods.** We first characterized the monocyte population in peripheral blood (PB) of 26 CLL and 13 healthy donors by flow cytometry. Furthermore, we evaluated monocyte migration by using Transwell assays and up-regulation of interleukin-10 (IL10), IL8 and MMP9 by qPCR and ELISA assays. Finally, we compared the gene expression profiles between monocytes collected from PB of 5 CLL patients and 5 healthy donors by using whole-genome microarrays. **Results.** Significant increase in nonclassical CD4+CD16++ monocytic subpopulation was detected in CLL compared to normal controls (10.4%±1.1% vs. 7.0%±1.1%) (p=0.04). Moreover, CLL patients with high risk genomic abnormalities were characterized by increased nonclassical monocytes (13.6% ± 2.5% vs. 8.5% ± 1.0%) and decreased classical monocytes (74.7% ± 3.1% vs. 68.3% ± 2.8%) compared to low risk FISH CLL (p=0.038). In addition, CLL patients displayed increased number of circulating monocytes expressing Tie2 receptor (TEMs) compared to normal controls (84.5 vs. 39.9/µl respectively, p=0.02). TEMs in CLL patients with adverse genomic aberrations were increased in comparison to low risk FISH CLL (157.9 ± 50.2/µl vs. 57.1 ± 17.9/µl) (p=0.04). We also confirmed that TEMs were present in CLL-infiltrated lymph nodal compartment by immunohistochemical staining.We identified 65 genes significantly up-regulated and 48 genes down-regulated in CLL monocytes compared with monocytes from normal controls (FC=2, p<0.05). The most up-regulated gene in CLL-derived monocytes is the GTPase-activating protein RAP1GAP (FC=6.5), able to inactivate RAP1 protein involved in phagocytosis. Conversely, we found a 12- and 3-fold down-regulation of tubulins TUBB3 and TUBB2 as well as a 2-fold decrement of CDC42EP3, a Rho GTPase effector protein involved in actin assembly at nascent phagosomes. In addition, we detected a 3.8-fold down-regulation of the chemokine ligand 5 (CCL5) involved in M1 polarization and a 3.2-fold down-regulation of prostaglandin reductase 2 (PTGR2), which catalyzes the reaction for inactivating the immunosuppressive prostaglandin E2 (PGE2).Ultimately, we found that CLL cells were able to attract monocytes and to induce the up-regulation of IL10, a Th2-derived cytokine able to inhibit inflammatory response, IL8, a pro-angiogenic chemokine, and MMP-9, a proteolytic proenzyme involved in degradation of extracellular matrix.**Conclusions.** These alterations further contribute to characterize the complexity of factors potentially involved in acquired immune deficiency of CLL patients. Overall, our findings indicate that the CLL-mediated "education"of immune elements may also include the establishment of a dysfunctional, immunosuppressive, pro-angiogenic and invasive phenotype in monocytes/macrophages.

0125

CPG INDUCES SURVIVAL AND PROLIFERATION OF UM CLL CELLS THROUGH ACTIVATION OF MYC/MIR-17~92 AXIS

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Background. Chronic lymphocytic leukemia (CLL) cells from clinically aggressive cases (i.e. expressing unmutated, UM, IGHV) have a greater capacity to respond to external microenvironmental stimuli, including those transduced through Toll-like Receptor 9 (TLR9). In CLL, several studies has identified certain microRNAs as either implicated in disease pathogenesis, or as part of a microRNA signature predicting clinical outcome or drug resistance. However, little is known regarding the capacity of external stimuli to modulate the expression of specific microRNAs and/or microRNA families. **Aims.** To investigate the modulation of microRNAs upon TLR9 triggering in CLL. **Methods.** Freshly-isolated negatively-selected CLL cells from 16 CLL patients (8 with UM and 8 with mutated, M, IGHV) were stimulated with CpG (18 hours), or left unstimulated or stimulated with anti-IgM as further control. miRNA and Gene Expression Profiling (GEP) were performed and validated by qRT-PCRs and transfection of miR/antagomiR in primary negatively purified CLL cells. **Results.** Concomitant microRNA and GEP in CLL cells expressing either UM or M IGHV genes, selected microRNAs from the *miR-17~92* family as significantly upregulated and responsible for modifications in the gene expression profile in CpG-stimulated UM-CLL, but not in M-CLL cells. In addition, the *miR-17* gene regulator *MYC* and the MYC target genes *CAD*, *PGK1* and *TFAM* were also upregulated in CpG-stimulated UM-CLL cells. Notably, neither the *MYC*/*miR-17~92* pair nor the MYC target genes were upregulated in UM/M CLL upon BCR triggering. Time-course experiments using primary UM-CLL cells showed that *MYC* and *miR-17* were upregulated by CpG stimulation following different kinetics: a rapid and transient induction for *MYC*, and a slow and sustained up-regulation for *miR-17*. These findings, indicate an associative interactions between *MYC* and *miR-17~92* in CLL, as described in other cell systems. Negatively purified UM-CLL cells transfected with pri-miR-17 showed a significant increase in *miR-17* expression levels compared to scrambled control (*P=0.003*), along with the down-regulation of *miR-17* target genes. By evaluating these cells for apoptosis and viability in serum-deprived cultures, *miR-17*-transfected UM-CLL cells showed a higher percentage of viable cells, already evident at 24 hours (*P=0.021*) and peaking at 48 hours (*P=0.010*) of culture. In complementary experiments, purified UM-CLL cells were transfected with a mixture of antagomiRs against *miR-17*, *miR-18a*, *miR-20a* and *miR-20b* or scrambled sequences, as negative control. In samples transfected with the negative control, CpG-stimulation increased the levels of *miR-17~92* microRNAs and induced leukemic cell proliferation, as evidenced by BrdU incorporation. The antagomiR mixture inhibited the CpG-induced increase of microRNA levels and concomitantly reduced the percentage of BrdU-positive CLL cells. The *miR-17* expression level, analyzed in a series of 83 CLL, was significantly higher in UM CLL (*P=0.032)*, in ZAP-70+ CLL (*P=0.026*), or UM/ZAP-70+ CLL (*P=0.015*) compared to their relative counterparts. **Conclusions.** These data reveal a role for the axis *MYC*/*miR-17~92* family in regulating pro-survival and growth-promoting responses of UM-CLL cells to TLR9 triggering and indicate that TLR9 and BCR triggering operate through different non-redundant stimulation pathway.

0126

TUMOR DYNAMICS OF CHRONIC LYMPHOCYTIC LEUKEMIA CASES WITH MULTIPLE PRODUCTIVE IGHV-IGHD-IGHJ REARRANGEMENTS

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Background. Due to the currently routine testing of IGHV mutational status in chronic lymphocytic leukemia (CLL) patients, evidence of cases with multiple productive immunoglobulin heavy genes rearrangements (MP-IGH) has

emerged. Several mechanisms, such as lack of allelic exclusion at the IG loci, or the presence of two clonal populations have been linked with the MP-IGH phenomenon. However, the true biological and clinical implications are currently unknown. Moreover, over-time clonal drift, a dynamic process of alterations in malignant clones recently observed in other lymphoid malignancies, has not been studied in these cases. **Aims.** Our study aimed to obtain molecular insight into the biological causes of MP-IGH in CLL and to perform a systematic study of the clonal drift in such cases. **Methods.** Separated B-lymphocytes from peripheral blood of 31 patients with MP-IGH were obtained. IGHV-IGHD-IGHJ, IGKV-IGKJ and IGLV-IGLJ rearrangements were analyzed from cDNA following described protocols. In available samples, PCR was repeated using gDNA; partial IGHD-IGHJ gene rearrangements and KDE rearrangements inactivating IGK loci were assessed. Detailed immunophenotypization was also performed. For the molecular monitoring of clonal dynamics, allele-specific oligonucleotide PCR assays (ASO-qPCR) were used. **Results.** Thirty one CLL cases with MP-IGHs were analyzed (two or three IGH rearrangements were detected in 26 and 5 patients, respectively). Firstly, we sought to unravel the possible causes underlying the presence of MP-IGH by performing a detailed immunophenotypic and molecular profiling. By coalescing all such results, we categorized the MP-IGH cases as follows: (i) definite co-existence of two clonal B-cell populations: 9/31 cases, with differing immunophenotypic light chain restriction; (ii) highly likely co-existence of more clonal populations with homogenous phenotype: 16/31 cases, where the number of detected IG rearrangements exceeded genomic capacity of single cell IG loci; (iii) indeterminate as to one or more CLL-like populations: 6/31 cases, in which we failed to obtain conclusive evidence of more than one clone. Furthermore, repeated MP-IGH analysis was performed in 23/31 patients (median interval from first to last analysis 24 months, range 8-74 months) to evaluate over-time changes in proportion of clones. Based on fragment analysis, detected IGH were considered as (i) persistent (stable in subsequent samples), (ii) appearing (originally undetectable), or (iii) diminishing (decreasing proportion in subsequent samples). In 11/23 patients, ASO-qPCR was used to confirm and quantify such changes. Interestingly, we observed clonal drift in MP-IGH cases: appearing of a new clone in three patients; diminishing of a clone in 18 patients. Moreover, analysis of molecular features of detected IGH revealed that selection of IGH tends to favour (i) higher IGHV identity to germ-line and/or (i) longer HCDR3. This tendency was more striking in cases where stereotyped BCR was selected.**Summary**: Our study allowed us to obtain novel insight into the biological causes of MP-IGH in CLL by categorizing patients according to the evidence of more than one B-lymphocyte clones. Importantly, we observed clonal drift favouring specific BCR molecular features, which might eventually provide further insight into disease evolution with implications for patient monitoring and therapy. Supported by CZ.1.05/1.1.00/02.0068, CZ.1.07/ 2.3.00/20.0045, CZ.1.07/2.4.00/17.0042, MSM0021622430, MUNI/A/0784/ 2011, GACR204/09/H058.

0127

PHOSPHOPROTEOMIC ANALYSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA AND B-CELL RECEPTOR SIGNALING SUGGESTS NOVEL THERAPEUTIC TARGETS

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Background. Chronic Lymphocytic Leukemia (CLL) is currently incurable using conventional therapies. CLL is similar to many malignancies in its dependence on microenvironmental survival signals, but almost unique in that CLL appears to utilise antigen as a pro-survival ligand. Studies suggest that antigenic ligation of the B-cell receptor (BCR) plays a fundamental role in disease pathogenesis. Few consistent BCR signaling abnormalities have been noted. **Aims.** We sought to examine BCR signaling abnormalities in CLL by comparing with signaling in healthy B-cells. BCR signaling differences via IgM and IgD isotypes were also sought. We used the high-throughput method of mass-spectrometry (MS) based phosphoproteomics to analyse multiple signaling pathways simultaneously to provide an overview of active signaling pathways without *a priori* knowledge of which pathways to investigate. **Methods.** Apoptosis assays, calcium flux and western blotting were performed after BCR crosslinking using $F(ab')_2$ fragments to IgM or IgD. The BCR of CLL and tonsillar B-cells were stimulated for 5 minutes followed by lysis, peptide digestion, enrichment for phosphopeptides (typically 75%) using TiO_2 beads and analysis using HPLC-MS/MS (Orbitrap). IgD and IgM signaling was compared, and signaling in 6 CLL (3 *IGHV* gene mutated, 3 unmutated) was compared to BCR signaling in 4 control healthy tonsil B-cells.4. **Results.** 4,575 unique phosphopeptides were identified and quantified using a label-free technique based on extracted ion currents. Phosphosite quantifications were compared between CLL and tonsil samples, at baseline and after stimulation. 174 phosphoproteins ($p<0.001$, fold change up to >4000-fold) were overexpressed in CLL relative to healthy B-cells. These included components of RNA processing complexes, cytoskeletal regulators and MAPK signaling pathway components. Kinase prediction based on phosphoprotein substrates confirmed activation of kinases known to be active in CLL (such as AKT1, ERK1/2, casein kinase 2), but several novel kinases (such as CaMK1, CRIK, ROCK1 and BCKDK) were also active in CLL relative to healthy controls.Evaluation of differentially expressed phosphoproteins after BCR ligation suggested a limited number of phosphosites altered by BCR signaling. These included components of the spliceosome, regulators of the cytoskeleton, as well as known BCR signaling components. Kinase prediction suggested high constitutive activity of ERK and LYN kinases, consistent with their known roles in CLL, and BCR-induced kinase activities included CDK family members, MAPKs, BCKDK and others.Finally, incubation of the PI3 kinase inhibitor CAL101 resulted in different drug sensitivities in the 6 leukemia samples, the degree of apoptosis only partly correlated with AKT1 activity (a downstream marker of PI3K activity).5. **Summary and Conclusions.** Mass-spectrometry based phosphoproteomics is beginning to offer powerful methods for interrogating intracellular signaling, with networks of phosphorylation characterising the topology of pathways. BCR signaling in healthy B-cells has not previously been studied using this approach, and comparisons with CLL highlight known pathways as well as suggesting novel treatment targets. The goal is to identify kinases active in CLL that will provide rational and effective drug combinations.

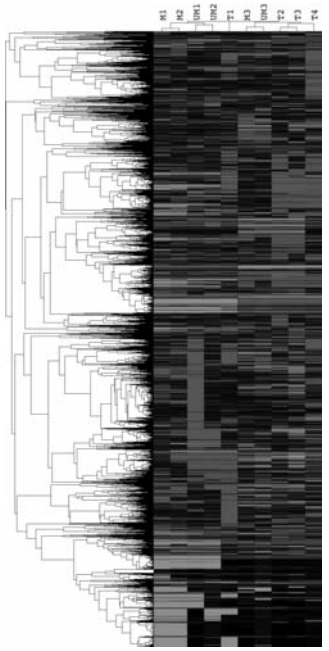

Figure 1. Unsupervised hierarchical clustering of phosphopeptides in unstimulated cells.

0128

MUTATION STATUS OF ATM GENE IN CLL PATIENTS HARBORING DELETION 11Q OR TP53 DEFECT

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Background. Abnormalities of *ATM* gene are frequent in chronic lymphocytic leukemia (CLL) patients and represent important predictive and prognostic factor. *ATM* defects are commonly assessed through monitoring of 11q deletion (11q-) using I-FISH. However, there are two aspects which may hamper setting aside affected patients properly: (i) 11q deletion does not mean ATM inactivation if the other allele remains intact (ii) there are patients who harbor *ATM* mutation without accompanying 11q-. Mutation analysis of *ATM* is complicated due to the extreme gene size and lack of preferential (hot-spot) mutations. Nevertheless, knowledge of *ATM* mutation status should significantly improve patient stratification. **Aims.** The aims were to establish an efficient and convenient system to detect *ATM* mutations and assess their frequency in high-risk CLL patients. **Methods.** We used the following complementary methodologies: (a) resequencing microarray (Affymetrix platform), which was designed to detect

1-nt substitutions (i.e. missense mutations, nonsense mutations, and substitutions in splicing sites) (b) western blotting (WB) to disclose patients with null ATM protein (c) functional testing based on induction of *CDKN1A* (p21) and *BBC3* (PUMA) genes after treatment of CLL cells with fludarabine and doxorubicin in parallel; in case of *ATM* mutation, the former drug leads to the gene expression induction, while the latter does not. **Results.** The resequencing on microarray was performed in 107 CLL patients. We detected 16 *ATM* mutations (13 missense, 2 nonsense, and 1 splicing) in 15 patients (14%). In parallel analysis, 11 out of 107 patients (10%) showed null ATM protein on WB; among these patients, there were six with mutation detected on microarray, two patients with three mutations together identified by direct Sanger sequencing only (two short deletions, one missense), and in remaining three patients a suspect mutation is still under investigation by direct sequencing. In total, there were 20 patients (19%) with demonstrable ATM defect (presence of mutation and/or null protein level). Our third test consisting of the ATM functional assessment (see Methods) indicated mutation in 12 patients; in 9 cases the mutation has already been identified. Not all identified mutations in our study, however, resulted into "ATM dysfunctional status". Proportions of patients with ATM defect in subgroups divided according to the presence of high-risk genetic features were following: 6 % (3/49) in patients having *TP53* mutation and/or deletion 17p; 32 % (13/41) in patients with 11q-; 24% (4/17) in patients without any adverse genetic defect. This observation confirms the previous data showing an exclusivity of *ATM* and *TP53* defects (although not strict) and suggests that the frequency of *ATM* mutations may not be dramatically different between the subgroups with or without 11q-. **Summary and Conclusions.** Our data confirms that *ATM* mutations do not automatically overlap with 11q- in CLL patients and are rare in *TP53*-defected subgroup. Several complementary methodologies should preferably be used to effectively assess *ATM* status. The work was supported by grants NS 9858-3, MUNI/A/0784/2011, and CZ.1.07/2.3.00/20.0045.

0129

DIFFERENTIAL REGULATION OF NOTCH ISOFORMS AND INDUCTION OF APOPTOSIS IN CLL CELLS BY GLIOTOXIN

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Background. Members of the NOTCH gene family (NOTCH1-4) encode highly conserved transmembrane receptors that modulate many cellular differentiation processes and are also involved in tumor initiation and progression. We and others have reported that CLL cells express constitutively activated NOTCH2 which is associated with enhanced cell viability. Moreover, gain of function mutations in the NOTCH1 gene were found in a subset of CLL patients suggesting a significant role for NOTCH1 and NOTCH2 in the pathogenesis of CLL and as potential therapeutic targets. However, the clinically relevant class of NOTCH regulators, ie. γ-secretase inhibitors, appears to be only effective in a small proportion of CLL cases *(Hubmann et al; BJH 2010)*. In addition, there is no information on the expression and regulation of the other NOTCH family members in CLL. **Aims.** The aim of this study was to explore the effect of the NOTCH2 transactivation inhibitor gliotoxin (WO 2006/135949) on the viability of CLL cells and how gliotoxin regulates other NOTCH family members. **Methods.** To maintain NOTCH2 activity *in vitro*, CLL cells were cultured in suspension in the presence of PMA (*PMA model*) or under co-culture conditions using primary bone marrow stromal cells (BMSC) *(Microenviroment model; Shehata et al., BLOOD 2010).* The nuclear NOTCH activity was analysed by electrophoretic mobility shift assays (EMSA). FACS analysis was used to quantify the surface expression of the NOTCH2 target gene CD23 and to evaluate apoptosis. RT-PCR experiments were performed to analyse the effect of gliotoxin on the transcription of NOTCH and apoptosis related genes. **Results.** Gliotoxin completely blocked the formation of DNA-bound NOTCH2 complexes in all tested CLL cases (n=21) at nanomolar concentrations after 24 hours of incubation. The inhibition of NOTCH2 signaling by 200 nM gliotoxin was associated with induction of apoptosis (mean: 67±31 SD versus 13±14 SD) and downregulation of *CD23A* transcription and CD23 surface expression (mean: 42±32 SD versus 83±17 SD). Exceptionally, one CLL case with a recently described *NOTCH1* gain of function mutation was found to be less sensitive to gliotoxin. Short term (4 hours) exposure of CLL cells revealed that the induction of apoptosis by gliotoxin is independent of NFκB activity and the expression of the antiapoptotic genes *BCL2* and *MCL1*. Exposure to gliotoxin resulted in downregulation of *NOTCH1*, *NOTCH2* and *NOTCH4* mRNA expression. In contrast, gliotoxin induced the expression of *NOTCH3*, its target gene *HEY1,* and *NR4A1* (activation induced cell death mediator). In addition, giotoxin selectively abolished the supportive effect of BMSC on CLL cells under co-culture conditions. **Summary and Conclusions.** The data show that CLL cells differentially express all NOTCH family members and that gliotoxin is an efficient NOTCH isoform modulator in CLL cells. The induction of *NOTCH3, HEY1* and *NR4A1* expression by gliotoxin reveals the complex role of different NOTCH genes in the regulation of apoptosis in CLL cells. Thus, gliotoxin may have a potential therapeutic value and a beneficial effect in CLL patients which warrants further evaluation.

0130

450K DNA METHYLATION ARRAY ANALYSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA REVEALS GLOBAL METHYLATION TO BE RELATIVELY STABLE OVER TIME AND SIMILAR IN CLL CELLS DERIVED FROM DIFFERENT ANATOMICAL COMPARTMENTS

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Background. The advent of global investigations has begun to unravel the functional involvement of DNA methylation in leukemogenesis. Previously, we identified divergent chronic lymphocytic leukemia (CLL) subgroups, IGHV-mutated and unmutated CLL, to have differential methylation patterns effecting key canonical pathway, proliferation and apoptotic genes. Despite these advances, in CLL cells, little to no knowledge exists regarding the extent to which DNA methylation changes with respect to time and exposure to different microenvironments. **Aims.** Our specific aims were, to compare the global DNA methylation profiles of IGHV mutated and IGHV unmutated CLL patients, to asses DNA methylation changes over time and investigate DNA methylation in CLL cells derived from different anatomical compartments. **Methods.** Using high-resolution 450K DNA methylation arrays, the DNA methylation profiles of 9 IGHV-mutated and 9 IGHV-unmutated peripheral blood diagnostic samples were analyzed. A second follow-up sample derived from these same patients collected ~5-8 years later, were additionally analyzed to study DNA methylation over time. Between the first and second sampling time-points, IGHV-unmutated patients were treated, whilst IGHV-mutated patients remained untreated. To study the effect of the microenvironment on CLL cell DNA methylation, 10 patient-matched peripheral blood (PB) and lymph node (LN) samples were also investigated. **Results.** On a larger scale to our previous 27K array study, we revealed 2239 CpG sites as differentially methylated between IGHV-mutated and unmutated CLL patients. Interestingly, the majority of sites were positioned outside CpG islands and promoters. Novel findings include differential DNA methylation of CpG sites within known differentially expressed CLL prognostic genes *CLLU1* and *LPL,* where methylation was notably higher in IGHV-mutated cases. Additionally, genes occupying TGF-ß and NF-κB/TNFR1 pathways and large numbers of polycomb target genes were shown to be differentially methylated. Over time, few large recurrent DNA methylation changes were noted among the subgroups. Although a larger number of non-recurrent differentially methylated sites were identified in IGHV-unmutated relative to IGHV-mutated cases over time, these changes equated to a low (<1%) global change. Similarly, few global recurrent and non-recurrent changes were identified between the patient-matched PB and LN compartment cases. **Conclusion**In summary, we confirm and extend the distinct methylation profiles of CLL subgroups. For the first time, we ascertain global DNA methylation to be relatively stable over time independently of treatment status, and demonstrate DNA methylation to be remarkably similar in CLL cells derived from different anatomical compartments.

0131

IMMUNOTOLERANT MOLECULE PROGRAMMED DEATH-1 IS A NOVEL HALLMARK OF ACTIVATED IMMUNOPHENOTYPE OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background. The programmed death-1 (PD-1) molecule is an immunoreceptor, which through an interaction with its ligand (PD-L1), controls peripheral tolerance by limiting activation, development and effector functions of T lymphocytes. PD-1/PD-L1 pathway was found to be one of the potential tumor escape mechanism from the immunosurveillance and its expression was described on exhausted T lymphocytes. **Aims.** We assessed a mRNA level and surface expression of PD-1 and PD-L1 in CLL patients since the aberrant expression of PD-1 and PD-L1 might represent a novel T-cell feature on the lymphocytic B-cells. It might also define a possible prognostic marker. In the functional studies, the PD-1 expression was investigated under conditions mimicking influence of microenvironment in germinal centers.**Patients and Methods.** Quantitative reverse transcriptase PCR (qRT-PCR) was performed for the PD-1 transcript in 182 CLL patients and 10 healthy volunteers (HVs). For four PD-1 splicing variants as well as for PD-L1 and one splicing variant qRT-PCR was made in 43 CLL patients. For characterization of the surface expression of PD-1 and PD-L1 on leukemic B-cells in a group of 45 CLL patients, magnetic separation of $CD19^+$ cells followed by five parameter flow cytometric analysis was used. In functional studies, cells from 21 CLL patients and 11 HVs were stimulated with IL-4 and CD40L, and analyzed for the expression of CD38 and PD-1 using flow cytometric afterwards. **Results.** The median level of PD-1 transcripts in CLL patients was higher in comparison with HVs (p=0.0176, n=182). Additionally, expression of a truncated PD-1 splicing variant lacking of exons 2, 3 and 4 was lower in CLL patients compared with HVs (p=0.0007, n=43). The PD-1 transcript level was comparable between PBMC (n=149) and BM (n=140) samples. No difference of PD-L1 expression between CLL patients and HVs on mRNA level was observed. In flow cytometric analysis, we confirmed the presence of PD-1 and PD-L1 on the CLL cells surface. Notably, both PD-1 and its ligand were expressed on the same CLL cells. Median PD-1 expression was higher on $CD5^+CD19^+$ cells of CLL patients in comparison to control B cells of HVs (47.2% vs. 14.81%, p<0.0001). Notably, levels of PD-1 on healthy B cells were similar in HV and in $CD19^+CD5^-$ B cells of CLL patients. Expression of PD-L1 was observed at similar levels in CLL and HVs. Interestingly, the mean fluorescence intensity values of PD-L1 reflecting the expression levels on CLL cells were higher when compared to HVs (p=0.017). There was no difference of time to progression and overall survival in groups of patients characterized by low and high PD-1 and PD-1L expression. After stimulation with IL-4 and CD40L, protein expression of PD-1 was significantly increased in samples that responded and up-regulated CD38 in comparison to not responding samples (p=0.0093). **Conclusions.** PD-1 is a novel immunotolerant molecule expressed on CLL cells on both mRNA and cell surface levels, additionally being up-regulated on CLL cells with the immunophenotype reflecting proliferative potential. The PD-1 might be involved in the pathomechanism of CLL and provide a novel target for future therapies.

0132

SYSTEMATIC EVALUATION OF LYMPHOCYTE SUBPOPULATION INBALANCES AND BONE MARROW CHANGES IN CLL PATIENTS TREATED WITH THE FCR REGIMEN: FOCUS ON CYTOTOXIC CELL EXPANSIONS AND ASSOCIATED DYSHEMATOPOIESIS

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A sizeable proportion of lymphoma patients treated with Rituximab-based regimens develop late onset neutropenia (R-LON) of as yet undefined cause(s). As we have previously shown, at least some R-LON cases may be attributed to autoimmune myelopathy/myelodyplasia arising in a context of cytotoxic T cell expansions with a large granular phenotype (CD3+CD8+CD57+). However, the retrospective character of all previous studies dealing with R-LON, including ours, created uncertainty as to whether the findings could be disease- and/or treatment-biased, as the relevant cohorts included cases with different diagnoses who were also treated with different CHI regimens. Here we present the results from the analysis of peripheral blood (PB) lymphocyte subpopulations and the detailed histopathological and immunohistochemical examination of bone marrow biopsy (BMB) samples of 23 CLL patients treated uniformly with the FCR regimen. The study group included 17 males and 6 females with a median age of 59 years (range, 29-72). Twenty-one cases received 6 FCR cycles, whereas the remaining 3 discontinued at cycle 5 because of Grade III hematologic toxicity. One month after the completion of the last FCR cycle, hemoglobin and platelet values were normal in all cases. In contrast, neutrophil counts were often low, with a median neutrophil count of $0.8x10^9$/l (range, 0.3-1.7). Neutrophil counts eventually normalized in all cases, however, 3/23 cases (13%) developed Grade III-IV LON at a median of 6 months after the completion of therapy. PB examination by 4-color flow cytometry (FACS) after the last FCR cycle identified residual CLL cells in 8/23 cases (35%). Eighteen cases were also systematically subjected to T cell subpopulation analysis with the following. **Results.** (i) T8 cells >1.0x109/l, 2/18 cases (11%); (ii) T4/T8 ratio <0.7, 7/18 cases (39%); (iii) >20% CD3+CD8+CD57+CD28- (T-LGL), 10/18 cases (55%): in cases with expanded T-LGLs, TRBV clonality assessment by flow cytometry did not reveal dominant epitopes. Notably, T cell subset imbalances were observed irrespective of whether the patients developed LON or not. BMB examination for assessment of response was performed in all cases after the last FCR cycle. The main findings are as follows: (i) minimal infiltration with small CD20-CD79a+CD5+CD23+CD3- B cells in 8/23 cases (35%), representing residual disease; (ii) mild-to-moderate infiltration by small CD3+CD5+CD45RO+CD20-CD79a- T cells in 19/23 cases (82%); (iii) hyperplasia of the erythroid and megakaryocytic series with concomitant dysplastic changes in 17/23 (74%) and 16/23 (69%) cases, respectively; (iv) hyperplasia or hypoplasia of the granulocytic series in 11 and 12 cases, respectively, with shift-to-the-left, always with less than 2% CD34+ cells, effectively ruling out the possibility of therapy-related myelodysplasia. In the three cases with R-LON, BMB examination was also performed at onset of neutropenia: 2/3 cases had hyperplasia whereas the remaining case had hypoplasia of the granulocytic series; all 3 cases had pronounced shift-to-the-left. In conclusion, our findings show that T cell subset imbalances are frequent after FCR treatment for CLL with variable effects on the hematopoietic marrow. Furthermore, they support the notion that R-LON may represent the end of a spectrum of dyshematopoiesis associated with immune-mediated myelodysplasia/myelopathy.

0133

MOLECULAR DISSECTION OF SIGNALS PROVIDED BY ACTIVATED T CELLS TO CLL CELLS INDICATES MAJOR ROLE FOR IL-21 AND CD40 IN CONTROLLING PROLIFERATION AND CHEMORESISTANCE

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Background and Aim. Chronic Lymphocytic Leukemia (CLL) cells accumulate in peripheral blood (PB) but proliferate in secondary lymphoid organs, where they display increased resistance to apoptosis. The signals involved in CLL proliferation and chemoresistance *in vivo* remain unknown to date. In the proliferation centra (PC), CLL B cells are in close contact with activated $CD40L^+$ $CD4^+$ T cells. *In vitro* stimulation of PB CLL cells with CD40L results in chemoresistance and an apoptotic profile resembling the LN situation, but generally fails to induce proliferation. Activated T cells and follicular helper T cells can also secrete IL-21, which has an essential role in providing help to healthy B cells. Here, we dissected the signals provided by activated T cells versus 3T40L-stimulation to CLL with respect to proliferation and drug resistance. **Methods and results.** CLL cells were purified from PB and cultured with autologous T cells activated with anti-CD3/anti-CD28 antibodies (Tact) resulting CLL proliferation which depended both on CD40L- and IL-21-signaling. Remarkably, gene expression profiling revealed that Tact or CD40L induced a highly similar gene signature in CLL cells. An overlap of 1242 differentially expressed genes compared to PB CLL indicated that CD40L-signals play a major role in the Tact system. *In vitro* stimulation with only 3T40L+IL-21 also resulted in proliferation of CLL cells. Surprisingly, IL-21 abrogated the resistance induced by CD40L to certain drugs (fludarabine, roscovitine and bortezomib) but not to the BH3-mimetic ABT-737. Detailed analysis of the changes in the pro-/anti-apoptotic balance after IL-21 stimulation demonstrated this was due to a decrease in Bcl-2 and Bcl-xL expression. In addition, microarray analysis showed that 129 genes were differentially regulated by IL-21, among which were components of the JAK-STAT (STAT3), and apoptosis pathways (Bcl-2, Bcl-xL) as well as GZMB (Granzyme B). Of these genes, 80 were also different between 3T40 vs Tact stimulation. Finally, we wished to ascertain whether IL-21 is being produced *in*

vivo in CLL, and performed IHC stainings on paraffin LN samples from untreated patients, as well as Q-PCR analyses. Scattered among small lymphocytes, we observed IL-21 immunostaining in large cells. IL-21 mRNA was detectable in CLL LN samples, with highest levels comparable to *in vitro* TCR cell stimulation assays.These results indicate that activated T cells from CLL patients provide strong CD40L and IL-21 stimulation to CLL cells. This combination leads to proliferation and specific changes in gene expression, related to migration and susceptibility to apoptosis. If this scenario is representative of the situation in LNs, this would imply that proliferating CLL cells are more susceptible to certain drugs than the bulk of resting cells, which are protected by interaction with stromal cells. Lastly, we uncovered stong indications for expression of IL-21 in CLL LN tissue. **Conclusions.**These results are not only important for understanding the biology of CLL but might also open new treatment venues.

0134

NON-RECURRENT GENETIC ABERRATIONS IN CHRONIC LYMPHOCYTIC LEUKAEMIA: RELATIONSHIP TO RECURRENT GENETIC ABERRATIONS AND CLINICAL BEHAVIOR

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Background. Recurrent genomic aberrations like del13q14, del11q22, del17p13 and trisomy 12 are well described as important prognostic parameters in CLL and therefore routinely tested in the diagnostic -work-flow of CLL. The prevalence and prognostic relevance of additional recurrent and non-recurrent genomic aberrations as defined by higher resolution technology still needs to be investigated. **Aims.** To detect the most frequent additional and novel non-recurrent genomic aberration using high resolution SNP arrays and to correlate their effects to the different groups of CLL recurrent aberrations. **Methods.** We analyzed copy number alterations (CNAs) in 240 consecutive CLL patients using high resolution Affymetrix SNP arrays. To determine the importance of small, tumor associated genomic imbalances and loss of heterozygosities (LOHs) we performed matched pair analyses of CD19+ B-cells and PB neutrophils in 120 patients. **Results.** In total, 407 CNAs were detected in 213 patients (89%) and among these, 203 (85%) had at least one of the recurrent aberrations. However, 77 patients (32%) had non-recurrent CNAs in addition to the recurrent aberrations. Among 166 non-recurrent CNAs, losses (66%) and gains (34%) were distributed with different frequencies among recurrent groups: 25%, 42%, 92%, and 93% in the group with del13q14, trisomy 12, del11q22 and del17p13, respectively. The median was 0.73 of non-recurrent CNAs per patient with the range from 0.35 in the group of CLL with del13q14 to 2.9 in the group with del17p13. The most frequent non-recurrent CNA is the gain of 2p found in 13 patients (5%) mostly with unmutated VH genes (10/13). Consistent with previous reports, gain 2p was exclusively found in combination with one of the recurrent aberration, del13q14 (n=5), del11q22 (n=3), del13q/del11q22 (n=3), del17p13 (n=2) strongly indicating disease evolution. A common minimal 3.5 Mb gain of 2p16 spans the *REL* and *BCL11A* oncogenes, implicating their importance in the pathogenesis of CLL. Moreover, novel non-recurrent aberrations were discovered in 10 CLL cases (4%). Those aberrations mostly affect chromosomal regions, for example del14q23.1-q32 and gain 17q21.33-q22, that overlap with non-recurrent CNAs occuring in combination with the recurrent lesions. Seven tumor associated, copy number neutral LOHs present in CD19+ cells, but not in PB neutrophils were identified in 6 CLL patients. Although tumor associated LOHs are a rare event, they span chromosomal regions (11q, 13q, 19p) that may harbor novel imprinted genes or loss-of-function alleles with importance for the pathogenesis of CLL. **Conclusions.** In conclusion, comprehensive screening for non-recurrent genomic aberrations in CLL by high-resolution SNP array analysis reveals the genomic complexity of CLL and suggest that some of the most frequent non-recurrent aberrations like gain 2p16 and del14q23.1-q32 should be included into the standard diagnostic FISH panel for genetic testing of CLL in order to determine their prognostic significance.

0135

THE ROLE OF STROMAL FIBROBLASTS IN THE REGULATION OF TUMOR MICROENVIRONMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Compelling evidence supports the pivotal role of stroma, or microenvironment, derived signals in tumor progression. Chronic lymphocytic leukemia (CLL) cells *in vivo* evade apoptosis which might depend on their ability to secrete molecules that favorably manipulate the surrounding microenvironment. CLL cells occur throughout the hematopoietic system including the bone marrow (BM) where they have an opportunity to interact with stromal cells, such as fibroblasts (FB). Studies suggest that these FBs are neither part of the malignant clone nor of hematopoietic origin. We hypothesize that BM FBs might be functionally altered and play a role in microenvironmental fine-tuning through differential gene expression. Previous studies have highlighted the role of chemokine signaling in the regulation of the microenvironment. We hope to identify mechanisms other than chemokines that favor the survival of CLL cells. **Aims.** We hypothesize that similar to other malignancies, genetic changes occur not only in the cancer cells, but in the interacting stromal cells as well. By characterizing putative changes in stromal cell gene expression between CLL patients and control subjects we aim to identify molecular events that can be targets of therapeutic interventions. **Methods.** BM aspirates from well-characterized CLL patients (total n=125) and healthy controls (total n=10) were used to establish *in vitro* primary FB cultures. Confluent cultures were split three times. Total RNA was isolated from 14 patients and 10 controls. Samples were hybridized to the Agilent SurePrint G3 Human GE 8x60K microarrays. After background correction and normalization of the data, differentially expressed genes between patient and controls were identified by applying random variance model t-test using BRB-Array Tool software. Network, functional and canonical pathway analyses of differentially expressed genes were performed using Ingenuity Pathway Analysis software. The most up- or downregulated genes were validated using real-time qRT-PCR on total RNA from arrayed samples. **Results.** Expression levels in patients were either increased or decreased at least 1.5-fold [range 6.8-(-5.8); $p \leq 0.05$] for over 500 genes as compared to those in controls. A set of chemokines and chemokine ligands were among the most upregulated genes in patients. In line with this, canonical pathway analysis identified chemokine signaling to be a significant differentiating factor between patients and controls. There were several non-chemokine genes demonstrating strong up- or downregulation in patients. Interestingly, among these was a member of the RUNX gene family, known for its oncogenic potential, showing significant downregulation (-2.4-fold; $p=1.97 \times 10^{-4}$) in patients. Among the classes of significantly altered biological functions were cellular movement, growth and proliferation; cell-cell signaling and interaction; and cell death. Validation data produced by real-time qRT-PCR were in keeping with the array results.**Conclusions.** We have shown that stromal FBs derived from CLL patients have different gene expression profiles compared to controls. In agreement with previous reports, pathway analysis suggested differences in chemokine signaling to be important, whereas functional analysis identified genes involved in cell-cell interactions. The functional mechanisms of the genes and pathways involved in the survival of CLL B-cells remain to be studied by *in vitro* experiments and in coculture with BM FBs.

0136

THE CYTOMEGALOVIRUS PROTEIN PUL32, WHICH IS RECOGNIZED BY B-CELL RECEPTORS ENCODED BY THE 1-69 GENE IN CLL, IS HIGHLY CONSERVED AMONG CLINICAL VIRUS STRAINS

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Background. Recently, we could show that recombinant antibodies (rAbs) of chronic lymphocytic leukemia (CLL) patients, encoded by the IGHV-1-69 gene, bind to the cytomegalovirus (CMV) large tegument protein pUL32 (pp150) (1). pUL32 is a highly immunogenic CMV protein with multiple epitopes dispersed over the 150 kDa protein. Analogous to other CMV antigens, host immune pressure to generate genetic diversity in the UL32-gene could be expected. Particularly CMV strains in CLL patients might be considered to be under strong selective pressure through a postulated persistent interaction of pUL32 with certain leukemic clones (1). The available information on the genomic diversity of this gene among clinical strains, however, is very limited. **Aim**. To determine UL32 genomic diversity in a clinical setting. **Methods.** We screened 200 consecutive

CLL patients for the presence of CMV-DNA and sequenced the UL32 gene to determine the genomic diversity among clinical CMV strains. Results were analyzed with respect to CMV-seropositivity and sequence of the Ig expressed on leukemic cells. As references, CMV UL32 sequences detected in patients with primary CMV infection (n=5) were used, in additional CLL patients treated with an anti-CD52 antibody (n=4), or sequences previously studied and available via PubMed (n=14). **Results.** CMV-DNA was detected in 3% and CMV-specific IgG antibodies in 71.5% of the 200 CLL patients. Interestingly, CMV-DNA was detectable in 2 CMV-seronegative patients. IgHV gene usage was associated neither with detection of CMV-DNA nor with CMV-seropositivity. Phylogenetic analysis of a total of 28 UL32 sequences, including 5 sequences from CLL patients, revealed low sequence variability (<1%). Moreover, the variability of UL32 observed between clinical strains was not restricted to specific stretches of the gene, rather it was uniformly distributed over the entire analyzed sequence. **Summary and Conclusions.** In contrast to other CMV antigens, such as glycoprotein B, pUL32 was found to be highly conserved among clinical CMV strains, including those from CLL patients. The function of pUL32 thus appears to be essential for CMV, considering the low genomic divergence of UL32 despite a presumed strong host immune pressure. The observed binding activity of distinct CLL rAbs to the same protein may hence be based on binding to different epitopes of the same protein.

Reference

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0137

REARRANGEMENT OF NOTCH1 OR BCL3 CAN INDEPENDENTLY TRIGGER PROGRESSION OF CLL

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Background. Recent data indicate that *NOTCH1* mutations significantly increase the risk of CLL progression towards Richter syndrome (RS) and chemoresistance, and that activation of *NOTCH1* at time of CLL diagnosis is an independent prognostic factor of poor survival. **Aims.** Our study aimed at identification of genetic defects triggering two consecutive progression events observed in a patient with CLL during 8 years of follow up. **Methods.** The applied techniques included conventional cytogenetic analysis, FISH, aCGH, qRT-PCR, Western blotting and Sanger sequencing. **Results.** The patient, a 58 year old male, was diagnosed with CLL (unmutated *VH*) in RS in June 2003. Karyotyping identified two related clones: (i) with an isolated +12 and (ii) with +12 and dic(9;14)(q34;q32). FISH and aCGH analysis showed that dic(9;14)(q34;q32) resulted in juxtaposition of 3'*IGH* and 5'*NOTCH1,* that was associated with loss of sequences distal to the involved loci. qRT-PCR analysis demonstrated a 10-fold upregulation of *NOTCH1* mRNA and a low expression of the neighboring genes (*GPSM11, CARD9, DNL2*). Immunoblotting of a cell lysate from lymph node with a NOTCH1 antibody recognizing active, cleaved NOTCH1 (Val1744), identified a band corresponding to activated intracellular NOTCH1. This finding suggested an additional truncating mutation that was further identified by sequence analysis. The detected two basepair deletion, ΔCT7544-7545/P2515fs, occurred in the nucleotide sequence encoding the PEST domain of NOTCH1. The patient was treated and achieved complete remission. In 2007, however, CLL relapsed and an examination of bone marrow (BM) identified a clone with a sole +12 negative for ΔCT7544-7545/P2515fs. Two years later, CLL progressed and BM revealed an evolved clone with complex aberrations including +12 and t(14;19)(q32;q13)/*IGH-BCL3* but lacking dic(9;14)(q34;q32). Of note, BM was again positive for ΔCT7544-7545/P2515fs. Despite treatment, evolving subclones with +12 were seen in the analyzed BM (06/2011) positive for ΔCT7544-7545/P2515fs. Four months later, the patient developed fatal peripheral T cell lymphoma. **Conclusions.** We deduced the sequence of multiple genetic defects driving development and progression of CLL in the presented case. An initial clone with +12, likely present at a presymptomatic CLL phase, later acquired an activating mutation of *NOTCH1*. Subsequent acquisition of dic(9;14)/*IGH-NOTCH1* triggered Richter transformation. After a few years, a persistent chemorefractory *NOTCH1*-mutated clone underwent another hit, t(14;19)/*IGH-BCL3*, which initiated the second progression that was followed by CLL-unrelated T-cell lymphoma. Our findings confirm the risk of activating mutations of *NOTCH1* in Richter transformation of CLL, particularly CLL with +12, and highlight that a residual chemotherapy-resistant *NOTCH1*-mutated clone is at risk of acquiring further progression-associated hits. Besides the known t(14;19)(q32;q13)/*IGH-BCL3,* we also identified dic(9;14)(q34;q32)/*IGH-NOTCH1* which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.

0138

VORINOSTAT (SAHA) INDUCED DOWNREGULATION OF C-FLIP CAUSE'S APOPTOSIS IN CD40L/IL-4 ACTIVATED CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. Chronic Lymphocytic Leukaemia (CLL) is the most common form of leukaemia in adults in the western world. First line chemotherapy treatment of CLL has prolonged first remission; however disease progression and chemotherapy resistance occurs. Studies have shown that the microenvironment is essential in maintaining CLL cell cycle progression and can contribute to drug resistance. Cellular Flice like inhibitory protein (c-FLIP) is a protein that has been linked to drug resistance. It inhibits the activation of caspase 8 thereby inhibits apoptosis. **Aims.** To examine if the pan-HDAC inhibitor Vorinostat (Saha) can induce apoptosis of primary CLL cells stimulated via CD40L/IL-4. **Methods.** CLL cell lines CII (UM) and PGA (M) were treated in a dose dependant manner and expression of c-FLIP isoforms are assessed by RQ-PCR and western blotting. Caspase inhibitors were used to investigate the dependency cell death mechanism. CD19+ cells were purified from peripheral blood of CLL patients by Miltenyl-automac, IGHV and cytogenetic status was determined. Cells were placed into a co-culture system with mouse fibroblasts (previously irradiated) expressing human CD40L and 10ng/mL IL-4 added. CLL primary Cells were treated with Saha in a dose dependant manner simultaneously or following previous CD40L/IL-4 stimulation. Samples assessed for apoptosis via Annexin V/PI staining. Expression of known pro-survival proteins was assessed using western blotting. Primary cells were treated with Fludarabine or Saha while in CD40L/IL-4 system and apoptosis assessed. **Results.** CLL cell lines showed downregulation of c-FLIP protein levels and activation of caspase 8 following Saha treatment. The accompanying cell death was shown to be rescued by pan-caspase and caspase 8 specific inhibitors but not with caspase 9 inhibitors. CD40L/IL-4 activated CLL cells showed significant upregulation of pro-survival proteins important in both the extrinsic and intrinsic death pathways, most notably Bcl-2, Mcl-1, Bcl-xL and c-FLIP. CLL (not in co-culture) cells treated with Saha showed a decrease in cell viability within 24hrs of initial treatment in a dose dependant manner with further decreased viability following r-TRAIL treatment. Additionally, primary CLL cells from 19 patients treated whilst in the CD40L/IL-4 system for 24hrs show significant levels of apoptosis. Furthermore, time course experiments showed that Saha inhibits the upregulation of c-FLIP when cells are in the co-culture system. Moreover previously co-cultured cells become apoptotic after Saha treatment. Finally Saha treatment alone of patient cells in CD40L/IL-4 show significantly more apoptosis compared to Fludarabine treatment alone. **Summary.** c-FLIP appears to be frequently upregulated in CLL patient samples with CLL (UM) cell lines showing higher levels. CD40L/IL-4 stimulation individually or in combination results in upregulation of expression of anti-apoptotic proteins including c-FLIP. Saha has been shown to downregulate c-FLIP expression and our results show stimulated/previously stimulated patients it inhibits upregulation. This results in reduction of CLL cell viability and an increase in apoptosis. These results indicate that Saha can overcome pro-survival interactions found in the CLL microenvironment and cause apoptosis through the resultant activation of Caspase 8. Therefore, Saha may be a potential therapeutic strategy for the treatment of CLL.

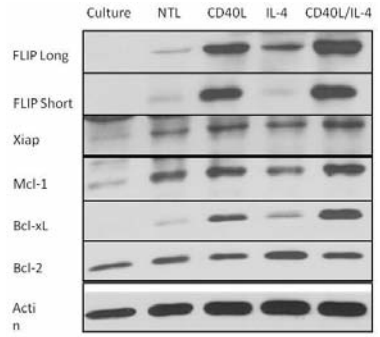

Figure 1. Combination of CD40L and Interleukin 4 on CLL primary cases causes synergistic upregulation of some pro-survival proteins compared to single.

0139

IDENTIFICATION OF INTERLEUKIN-4 (IL-4) TARGET GENES IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. Interactions of the malignant B lymphocytes with microenvironment play a crucial role in the pathogenesis of chronic lymphocytic leukemia (CLL). In this context, the cytokine interleukin 4 (IL-4) makes an essential contribution to B-cell differentiation and survival. In follicular lymphoma, a CLL-related disease, gene expression profile (GEP) studies suggest that a preeminent IL-4-dependent T-B cell axis sustains tumorigenesis. Activation of the IL-4 pathway might also contribute to CLL pathogenesis. In this study, the response of CLL cells to IL-4 in terms of GEPs was investigated. **Aims.** To identify the IL-4 target genes in CLL cells and normal B cells. **Methods.** Whole blood from 10 controls with normal lymphopoiesis and 10 chronic lymphocytic leukemia (CLL) patients was obtained prior informed consent. B cells were purified by negative isolation procedures. Aliquots of purified B cells were processed for total RNA obtention (Pre sample), or cultured for 12 hours in the presence of human recombinant IL-4 (IL-4 sample) or nothing (Ctrl sample). Two-color Whole Human Genome Microarrays (4 × 44k) were performed and analyzed with appropriate software for normalized extraction of data sets and statistics.Identification of IL-4 targets in CLL and normal B cells was performed using the 1-way ANOVA test ($p<0.05$) with posthoc Tukey HSD analysis. The IL-4 targets were found in the intersection between IL-4 vs Pre and IL-4 vs Ctrl comparisons. These analyses were followed by functional, pathway and network analyses. **Results.** Microarray analyses identified 203 IL-4 target genes in CLL (146 up, 57 down) and 343 IL-4 target genes in normal B cells (237 up, 106 down), being 84 genes common to both groups (76 up, 10 down). They included known and novel IL-4 targets. Top up-regulated genes in CLL were CCL17 (23-fold), RASL10A (21-fold), SLC24A3 (21-fold), HOMER2 (16-fold), SLC39A8 (11-fold), TLR7 (9-fold), QSOX1(9-fold), and CLDN1 (9-fold), and in normal B cells CCL17 (44-fold), IL4I1 (19-fold), RASL10A (15-fold), BATF (14-fold), IGSF3 (10-fold), MFI2 (9-fold), AHRR (8-fold), and TGM2 (8-fold). Down-regulations were weaker than up-regulations. Gene regulation by IL-4 was consistent with activation of STAT6 in both CLL and normal B cells, and NOTCH1 in normal B cells. In spite of the known pro-survival effect of IL-4 on CLL, confirmed by our own data, functional annotation of IL-4 targets predicted increased activation state of cell death in CLL and normal B cells (regulation z-scores of 1.998 and 1.861, respectively, near the cut-off level of 2). Significant canonical pathways for IL-4 targets included JAK/Stat and GM-CSF for both groups, signaling by CXCR4, phospholipase C, p70SK6, IL-8, IL-2, and RhoA for CLL, and Notch1 for normal B cells. **Summary and Conclusions.** GEP studies demonstrate that IL-4 is active in CLL cells and in normal B cells. However, the IL-4 targets identified in both groups partially overlapped, suggesting activation of common and divergent functional pathways in CLL and normal B cells. These results suggest that IL-4 stimulation in the CLL niche may sustain tumorigenesis.mariaj.alcaraz4@carm.es

0140

DELETION (13Q14.3) AND/OR MIR15A-16-1 DOWNREGULATION: AN ALMOST CONSTANT FINDING IN CHRONIC LYMPHOCYTIC LEUKEMIA ?

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Background. Del(13q14.3) is the most frequent deletion reported so far in B-CLL. It was recently shown that two micro-RNA genes (mir15a and mir16-1) are deleted in almost all cases carrying the deletion. However, large fluorescent in situ hybridization (FISH) studies fail to detect del(13q14) in up to 40-45% of B-CLL cases. Comparative genomic hybridization (CGH) technologies have substantially improved resolution, but are limited by noise, leading to mask small changes. **Aims.** To determine wether SNP random bead-arrays (Illumina, hybridization with oligonucleotides probes immobilized onto beads, that correspond to Single-Nucleotide Polymorphisms along the human genome, followed by single-base enzymatic primer extension) are more sensitive than interphase-FISH to detect del(13q14), or not. To discriminate between signal and noise, we also studied relationship between del(13q14) and mir15a/16-1 expression. **Methods.** A blood sample from 24 patients with B-CLL at diagnosis was analysed simultaneously using LSI D13S319(~130kb) (Abbott) and 13q14.3(~400 kb)/13q ter (Cytocell) FISH probes, SNP bead-array and miRNA expression. Blood samples from 24 healthy donors were used as negative controls. I-FISH: an average of 6% nuclei of normal lymphocytes showed loss of signal (truncated nuclei or random colocalized signals), and the cut-off to ascertain a chromosomal deletion was defined over 10% (m+3DS). SNP-array: 200 ng DNA was hybridized on the Illumina HumanCNV370 BeadChips, which assess ~373,397 markers (4.9 kb median marker spacing). miRNA: 200 ng total RNA was processed with Illumina's MicroRNA Expression Profiling Panel. **Results.** In 18 (75%) patients, del(13q14) was discovered using both I-FISH [13-95% nuclei; mean=47%; including 4 biallelic deletions] and SNP-arrays (size: 0.49-33 Mb). A clear-cut correlation was observed between percentage of I-FISH del(13q) nuclei and either SNP-arrays signals intensity [log*R* ratio and allele frequency (AF)] or reduced expression of mir15a/16-1 (greater amplitude in patients demonstrating biallelic deletion). In 5 other patients beadchips discovered del(13q) which was not detected after I-FISH, corresponding to cryptic del(13q14.3) (39-82 kb) in 3 patients (including 1 biallelic deletion) [displaying clear-cut correlation between SNP-arrays signals intensity and reduced mir15a/16-1 expression level]; and to (4-30Mb) deletions encompassing 13q14.3 in 2 patients with low clonal infiltration (respectively mono- or biallelic in 9% or 1% nuclei) [a clonal expansion was detected by I-FISH performed 6-12 months later]. In the last patient from this series del(13q) was undetected after both I-FISH and SNP-arrays, but mir15a and mir16-1 expression levels at <10% of normal cells were observed. **Summary and Conclusions.** Using BeadArray platform, we demonstrated del(13q14.3) and/or mir15a-16-1 downregulation in all B-CLL tested, even when FISH failed to detect an abnormality. Resolution and sensitivity as high as respectively 39 kb and 1% tumoral cells could be reached, with signals unambiguously discriminated from noise. We undertake a SNP-array study on a larger CLL cohort del(13q) negative by I-FISH. The presence of a deletion or another genetic abnormality located at 13q in all patients from this series leads to hypothesize a primary transforming lesion in lymphomagenesis, irrespective of prognostic features.

0141

TARGETING PROLIFERATING CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS WITH A NOVEL SYNTHETIC LOW DENSITY LIPOPROTEIN DRUG DELIVERY SYSTEM

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Background. Chronic lymphocytic leukaemia (CLL) currently remains incurable without stem cell transplantation, an option for only the minority of patients. Despite advances in chemotherapy, most patients relapse owing to the persistence of minimal residual disease (MRD). Substantial evidence has accrued to suggest that the tumour microenvironment is central to disease progression in CLL, with the bone marrow (BM) and lymph nodes (LN) acting as sanctuary sites for MRD. Whilst peripheral blood CLL cells are cell cycle arrested, significant rates of clonal proliferation occur in the BM/LN wherein acquisition of deleterious cytogenetic abnormalities such as 17p deletion may arise. Further, CLL cells co-cultured *in vitro* on stroma with CD154/IL-4 to give a proliferative signal, are chemoresistant to first line therapies. As proliferating cells require lipids for membrane synthesis, we hypothesise that proliferating CLL cells will have greater requirement for low density lipoprotein (LDL) compared to circulating CLL cells, and also that of normal resting lymphocytes providing a potentially differential cellular property to attack. Proof of concept of drug-loaded synthetic (s)LDL nanoparticles has been provided in glioblastoma and CML. We propose that drug loading into sLDL nanoparticles will allow selective targeting of proliferating CLL cells within the BM/LN proliferation centre, will protect drugs from plasma binding proteins, and will ultimately raise intracellular drug concentrations in the protective microenvironmental niche, to overcome chemoresistance. **Aims.** To determine (a) the extent of sLDL uptake by CLL cells compared to normal; and (b) whether sLDL uptake by CLL cells changes under proliferative conditions mimicking the proliferation centre. This will determine whether proliferating CLL cells have increased sLDL uptake compared to non-cycling CLL cells or normal B lymphocytes. **Methods.** sLDL uptake was assessed by flow cytometry, measuring the mean fluorescence intensity in the FITC channel owing to the stable incorporation of dioctadecyloxacarbocyanine (DiO) into the formulation. Internalisation was confirmed by deconvolution fluorescence microscopy. Primary CLL and normal donor samples were enriched for CD19+ B-lineage cells by magnetically activated cell sorting. Cells were cultured in media on tissue culture plastic or NT-L mouse fibroblasts with or without CD154/IL4. Lymphoid cells were stained with CellTrace Violet® to track cell division in response to proliferative signals (CD154/IL4 stroma). **Results.** HG3, a human lymphoblastoid cell line, avidly took up sLDL nanoparticles in a concentration (0-50 ng/mL cholesterol) and time (0.5-24h) dependent manner. Normal donor peripheral blood B-cells and CLL cells cultured on plastic did not actively take up sLDL but maintained their viability even in the highest concen-

tration sLDL tested. Actively proliferating CLL cells on CD154/IL4 stroma could be targeted with sLDL unlike their non-cycling counterparts; interestingly even the minor population of cells that had remained undivided on stroma were also found to be sLDL positive. **Summary**. CLL cells can be selectively targeted by sLDL nanoparticles with respect to their non-cycling counterparts. We next will investigate the *in vivo* targeting of sLDL which we hypothesise, by virtue of their size, will home to lymphoreticular organs, sanctuary sites for CLL MRD.

0142

IDENTIFYING NOVEL PROGNOSTIC BIOMARKERS IN CHRONIC LYMPHOCYTIC LEUKEMIA USING PROTEOMICS: A POSSIBLE ROLE FOR KININOGEN

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Background. Disease progression in Chronic Lymphocytic Leukemia (CLL) is characterized by a high degree of heterogeneity. Some CLL patients have an indolent disease course with normal life span and die of unrelated causes whilst other patients have an aggressive disease progression with comparatively rapid fatal outcome. The lack of predictive factors for disease progression is the catalyst for identification of reliable and affordable biomarkers. The hyper reactivity of the B cell Receptor (BCR) to unknown antigen stimulation is associated with malignant B-cell survival. The identification of novel protein expression which is correlated to BCR stimulation may have diagnostic, prognostic and therapeutic value. **Aims.** We aimed to employ proteomics to identify novel proteins associated with BCR stimulation in CLL samples. The expression of putative biomarkers would be correlated with clinical data in a large series of samples. **Methods.** Following ethical approval, blood samples were collected from CLL patients who had not received treatment within 3 months. Peripheral blood mononuclear cells (PBMCs) were extracted and stimulated by crosslinking the BCR with 10 µg/ml $F(ab)_2$ goat anti-human IgM $Fc_{5\mu}$ fragmentat 37ºC. Cells treated with isotype control were included as untreated control. Two-dimensional gel electrophoresis (2DE) with MALDI-TOF mass spectrometry (MS) was used to identify proteins associated with BCR stimulation in 3 CLL samples. Immunoblotting was used to confirm KNG overexpression in BCR-stimulated samples. Following this, a series of 52 CLL samples was screened for basal Kininogen (KNG) expression using immunoblotting. Survival and Time To First Treatment (TTFT) data were available for all CLL samples. Basal KNG expression was correlated with overall survival, TTFT and other prognostic biomarkers. **Results.** Proteomic analysis by 2DE/MALDI-TOF MS revealed upregulation of KNG after 5.5 hours of BCR stimulation in 3/3 clinical samples. Upregulation of KNG after prolonged stimulation was confirmed by immunoblotting in 4 samples, including 2 which were previously analyzed using proteomics. There are 2 major forms of KNG: low molecular weight KNG (LMWK) and high molecular weight KNG (HMWK). The peptides identified in MALDI-TOF MS best matched the sequence of LMWK. The analysis of basal LMWK by immunoblotting revealed positive expression in 69% (36/52) of CLL samples. No statistical significance was found with preliminary clinical correlations however there was a trend towards shorter median survival in LMWK positive cases (152 months as opposed to 264 months for LMWK negative cases) (p=0.161). **Conclusions.** Our study demonstrates for the first time that CLL B-lymphocytes express KNG and that the expression level differs between samples. KNG is an important protein of the Kinin-Kallikrein System and is the substrate for proteolytic cleavage of Bradykinin, which is a mediator of inflammation and pain and also facilitates the increase of vascular permeability. Further research into the expression of LMWK and HMWK and the association with BCR stimulation will determine the potential role of KNG as a prognostic biomarker in CLL.

0143

DETAILED MUTATIONAL ANALYSIS OF TP53 GENE REVEALS HIGH INCIDENCE OF ADDITIONAL MINOR PROPORTION MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background. Presence of aberrations in the tumor suppressor gene *TP53* is one of the strongest prognostic markers of chronic lymphocytic leukemia (CLL). Besides 17p deletion the individual *TP53* gene mutations were recently associated with unfavorable CLL prognosis. In addition to clonal *TP53* mutations, minor mutations may be detected in some CLL patients. Nevertheless, frequency and importance of mutations present in minor cancer clones wasn't investigated up to now. **Aims.** We employed sensitive *TP53* mutational screening to determine the incidence of minor mutations in CLL patients. **Methods.** For precise mutational testing the combination of functional analysis (FASAY) and denaturing high-performance liquid chromatography (DHPLC; Varian) in connection with direct sequencing was used. Selected samples were investigated by ultra-deep next generation sequencing (NGS; GS Junior System, Roche). **Results.** Mutational status of the *TP53* gene was assessed in 198 samples from 185 selected CLL patients (12 patients were analyzed repeatedly). We found 96 mutations in 63 patients. Notably, in 15 patients with major clonal mutation 20 minor gene mutations were clearly detected (1-3 mutations per patient). Among them, 10 missense mutations, 7 splicing mutations and 3 frameshift mutations were identified. The detected minor mutations were evaluated by ultra-deep NGS in 7 patients, and presence of all 11 tested mutations was confirmed (mutation proportion 3,24% - 6,62%). Interestingly, the patients with minor *TP53* mutations were more frequently pre-treated by chemotherapy in comparison with patients who carried a sole mutation (P = 0.0031). **Conclusions.** Clonal *TP53* mutations are often accompanied with additional minor mutations, particularly in patients after chemotherapy. Our results further support the role of treatment in selection of *TP53* mutations in CLL patients. Evaluation of clinical impact of these minor clones requires systematic long-term analysis of large patients' cohort.This study was supported by research proposal MSM0021622430 and projects CZ.1.05/1.1.00/02.0068, CZ.1.07/2.3.00/20.0045, CZ.1.07/2.4.00/17.0042, MUNI/A/0784/2011.

Chronic lymphocytic leukemia - Clinical 1

0144

INFLUENCE OF DIFFERENT TREATMENT REGIMENS ON SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA - A META-ANALYSIS OF THE GERMAN CLL STUDY GROUP (GCLLSG)

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Background. A variety of first-line and relapse therapy regimens for patients with advanced chronic lymphocytic leukemia (CLL) have been established in the past years. Though ESMO guidelines recommend to repeat first-line therapy if relapse occurs later than 24 months after first-line chemoimmunotherapy, these recommendations have not been confirmed by clinical trials so far. However, the most beneficial composition and sequence of regimens (antibody-based, chemoimmunotherapy, CHOP-like regimen) regarding patients´ treatment-free and overall survival remains unclear. **Aims.** The aim of this meta-analysis was to evaluate whether special therapeutic regimens have a positive impact on treatment-free and overall survival. **Methods.** From 1659 consecutive patients included in five different study protocols for first-line and relapse of the GCLLSG (CLL4, CLL5, CLL8, CLL2L (Fludarabine + Cyclophosphamide + Alemtuzumab; first-line and in relapse) and CLL2M (Bendamustine + Rituximab; first-line and in relapse) trial) we selected 1558 patients who received at least one therapeutic regimen. 101 patients had to be excluded never having received treatment in one of the trials. Patients were assigned to different treatment categories. For statistical analysis Kaplan-Meier estimators and curves were used including log-rank tests. **Results.** The median age at the beginning of first-line treatment of the whole series (n=1558, 1113M/425F) was 61 (30-81) years.At first, patients were stratified according to the first-line treatment they received. Comparing the different regimens according to the study generations of the last two decades (first generation: CLL 4 - fludarabine monotherapy vs. fludarabine + cyclophosphamide; CLL 5 - chlorambucil monotherapy vs. fludarabine monotherapy; second generation: CLL 8 - fludarabine + cyclophosphamide vs. fludarabine + cyclophosphamide + Rituximab) treatment-free and overall survival steadily increases along with the advances in clinical CLL research (see Figure 1a).Thereafter we focused on different treatment regimens that have been administered at any time during the course of disease. Patients who received an antibody-based regimen at least once during their therapeutic course (n=909) had a significantly longer overall survival than all other patients who had never been treated with antibody-containing therapy (OS after 60 months: 75.7% vs. 64.1%, p=0.006, see Figure 1b).

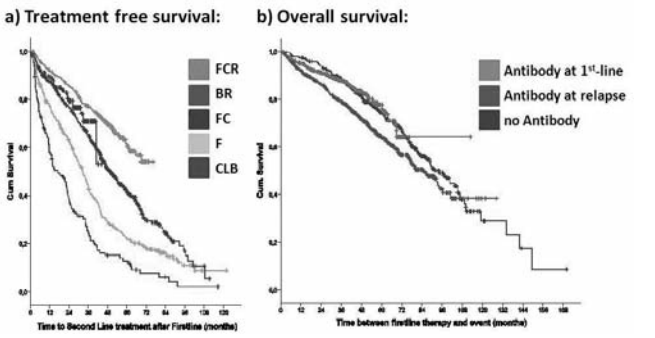

Figure 1. a) Treatment-free survival for patients with different first-line therapies b) OS of pts. with antibody-based regimen vs.pts.with antibody-free regimen.

This was independent from the time point of administration (first-line or relapse).The CHOP-regimen (Cyclophosphamide, Vincristine, Doxorubicine and Prednisolone), which was often chosen as relapse treatment due to its assumed efficacy especially in high-risk situations (early relapse, unfavourable prognostic markers), was used in 202 patients. The overall survival in the CHOP-collective was significantly shorter than in the comparative group ($p<0.0001$) although median observation time was not significantly different. However, this observation might reflect a bias in the selection of high risk patients for this relapse treatment. No influence on survival was observed in patients receiving a mitoxantrone-containing regimen at any time during the treatment course. **Summary and Conclusions.** This meta-analysis shows that the advances in the development of strategies for first-line therapies result in prolongation of treatment-free and overall survival for patients with CLL and need of treatment. Chemoimmunotherapies prolong the survival independently of the time point of chemoimmunotherapy administration (for first-line therapy or relapse).

0145

A SINGLE-ARM MULTI-CENTER TRIAL OF BENDAMUSTINE GIVEN WITH OFATUMUMAB (BENDOFA) IN PATIENTS WITH REFRACTORY OR RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA. GIMEMA CLL0809 PROTOCOL

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Background. The advent of chemo-immunotherapy associations has substantially improved the overall response rate (ORR), time to progression, and overall survival of chronic lymphocytic leukemia (CLL). However, CLL remains incurable and patients (pts) eventually relapse. The development of an effective therapy that is not cross-resistant with the treatment strategies currently available as front-line treatment is one of the clinical unmet needs in CLL. **Aims.** Within the GIMEMA network, we conducted a phase II, non-comparative, multicenter study (CLL0809) to assess the efficacy and safety of the combination of bendamustine and ofatumumab in relapsed/refractory CLL. **Methods.** Pts with active CLL, pre-treated with no more than two lines of therapy, were eligible. Therapy consisted of bendamustine (70 mg/m^2) for two consecutive days every 28 days and ofatumumab (300 mg on day 1 and 1000 mg on day 8 at the first cycle and 1000 mg on day 1 at the subsequent cycles). Treatment was administered up to 6 cycles. The response was assessed after 3 cycles and at the end of treatment. An extensive biological characterization was performed in all pts. **Results.** Fifty pts have been registered from 14 centers. One patient was ineligible due to active HBV infection. The median age was 66 years (46-81); 71% of pts were in Binet stages B/C and 30%had bulky adenopathy ≥ 5 cm. FISH analyses detected del(13q), del(11q), +12 or del(17p) in 47%, 12%, 20% and 20% of pts, respectively; 20% of pts carried a p53 mutation and 16% had NOTCH1 mutations; 59% of pts had unmutated IGHV genes,

whereas ZAP-70 and CD38 were positive in 65% and 43% of cases. Previous treatments were fludarabine, rituximab or alemtuzumab-based therapy in 71%, 53% and 12% of pts, respectively; 39% of pts were pre-treated with two lines of therapy.The response was assessable at the 3rd cycle in 35 patients and at the 6th cycle in 18 pts. The ORR was 89% at the 3rd cycle and 79% at the 6th cycle, with respectively 26% and 16% of complete remissions. At the 3rd cycle, pts with 17p- and/or p53 mutation had an ORR of 83%.Hematologic toxicity was the most common adverse event. Grade 3/4 neutropenia, thrombocytopenia and anemia occurred in 65%, 18% and 4% of pts. A total of 3 severe infections were reported, including 2 fatal sepsis. Grade 3 infusion reactions to the first administration of ofatumumab occurred in 4 pts (2 skin rash, 1 sinus bradycardia, 1 hypotension plus dyspnoea).Eight pts went off study: two pts did not start treatment (1 for lack of drug supply and the other for informed consent withdrawal); 3 pts died during therapy (2 deaths were due to sepsis, while 1 patient died owing to a duodenal ulcer hemorrhage); in the remaining 3 pts therapy was stopped because of severe neutropenia, acute myocardial infarction or increased troponine levels. **Conclusions.** This preliminary analysis shows that the combination of ofatumumab and bendamustine is feasible and substantially effective in relapsed/refractory CLL patients with high risk clinical and biological features.

0146

CHLORAMBUCIL FOR THE TREATMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) - SYSTEMATIC REVIEW AND META-ANALYSIS

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Background. Many trials assessing treatment for patients with CLL/SLL compares chlorambucil to different regimens, including other alkylating agents, purine analogues, and alemtuzumab. So far, none of these regimens improved overall survival as compared to chlorambucil, though improved response rates were shown with purine analogues. **Aims.** To assess the effect of chlorambucil on overall survival compared to other therapeutic regimens in patients with CLL/SLL requiring therapy. **Methods.** Systematic review and meta-analysis of randomized controlled trials that compared chlorambucil to other therapeutic regimens for patients with CLL/SLL requiring chemotherapy. We searched *The CochraneLibrary*, MEDLINE, LILACS, conference proceedings, and databases of ongoing trials. The primary outcome wasoverall survival. Secondary outcomes: complete response (CR) and overall response (complete and partial) rates, adverse events. Relative risk (RR) for dichotomous data and hazard ratio (HR) for time to event data were estimated and pooled. **Results.** We identified 18 trials recruiting patients between the years 1978 and 2006 randomizing 4662 adult patients, with previously untreated CLL/SLL. In 16 of the trials the mean/median age was less than 65 years. Indications for treatment were advanced disease (Binet stage B/C or Rai stage III/IV) or progressive disease at any stage. Chlorambucil was compared to fludarabine or cladribine; cyclophosphamide, vincristine, prednisone (COP); cyclophosphamide, adriamycin, vincristine, prednisone (CHOP); bendamustine; and alemtuzumab. Chlorambucil had a similar effect on overall survival as other chemotherapeutic regimens (HR 1.01, 95% CI 0.90 - 1.13, 15 trials, 4119 patients, random-effects model, Figure 1). Figure 1 depicts the effect of specific regimens compared to chlorambucil on overall survival. Sub-group analysis of overall survival showed a similar effect in different age groups, different comparators, and year of publication. Overall response rate did not differ between the allocated treatment groups. CR rate was inferior for chlorambucil compared to other chemotherapy regimens (RR 0.58 95% CI 0.36, 0.96, 4050 patients, 16 trials). Subgroup analysis of CR rate demonstrated that CR was inferior for chlorambucil compared to purine analogues, but not compared to COP or CHOP. Risk of grade 3/4 adverse events decreased with chlorambucil treatment (RR 0.59 95% CI 0.48 - 0.72, 3304 patients) as well as that of adverse events requiring discontinuation of chemotherapy (RR 0.45 95% CI 0.30 - 0.67, 3545 patients). **Conclusions.** Our systematic review showed with the perspective of time that chlorambucil remains a viable therapeutic option for patients with CLL/SLL due to similar effect on overall survival and overall response rate and a lower toxicity profile compared to other regimens in all age groups. Fludarabine containing regimen may be preferred in younger patients due to a higher complete remission rate. These results are limited by heterogeneity of the chemotherapeutic protocols, the wide range of study years, and the low quality of some of the included trials. None of the allocated treatments contained rituximab, which is currently the standard of care for first line treatment in CLL/SLL. Ongoing trials currently compare the effect of different chemotherapeutic regimens combined with rituximab to chlorambucil and rituximab.

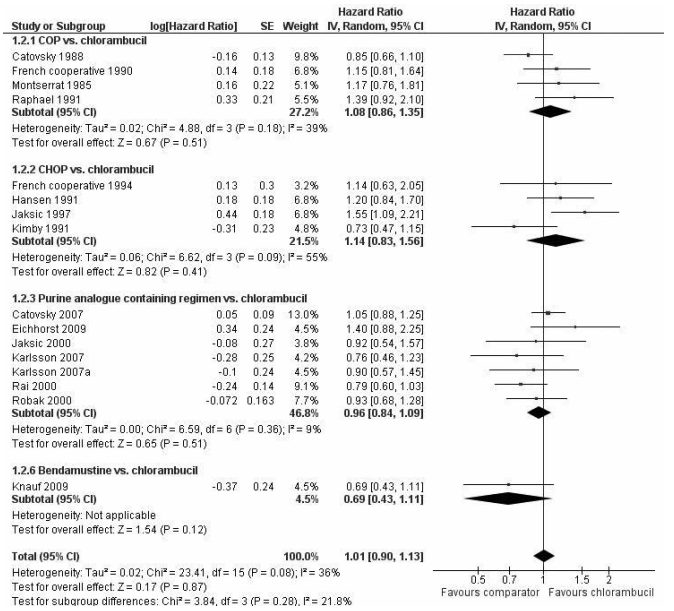

Figure 1. Effect of chlorambucil compared to other chemotherapeutic regimens on overall survival in CLL.

0147

NO BENEFIT FROM CHEMO(IMMUNO)THERAPIES CONTAINING ANTHRACYCLINES AND/OR =3 CYTOTOXIC AGENTS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) AND EARLY RELAPSE - A META-ANALYSIS OF THE GCLLSG

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Background. Despite a substantial improvement of treatment outcomes in patients with CLL due to development of more efficacious combination therapies and introduction of rituximab, almost all patients will eventually relapse. According to ESMO guidelines, repetition of 1st-line therapy is a reasonable approach only in case of a relapse >24 months after initial treatment (Eichhorst et al., Ann Oncol., 2011). Little is known which therapy should be chosen in case of an earlier relapse. **Aims.** The purpose of this analysis was to evaluate the outcome of different 2nd-line therapies after early relapse in order to identify potent 2nd-line regimen. **Methods.** A total of 1558 patients were treated within 5 trials of the GCLLSG. Three trials evaluated 1st-line therapies for physically fit (CLL 4 and CLL8) and elderly patients (CLL5), another 2 trials included 1st-line and relapsed patients (CLL2M and CLL2L). 315 of these patients required 2nd-line therapies within 24 months. **Results.** First-line therapies of these 315 patients included fludarabine, cyclophosphamide (FC, n=100), fludarabine (F, n=85), chlorambucil (Clb, n=62), FC with rituximab (FCR, n=34) or bendamustine and rituximab (BR, n=17).Second-line therapies applied in these early relapsing patients were quite heterogeneous. Common therapies were the combination of cyclophosphamide, doxorubicine, vincristine, and prednisolone either with rituximab (CHOPR, n=32) or without (CHOP, n=24), followed by alemtuzumab (A, n=27), FC (n=26), BR (n= 20), FCR (n=16) or FC

with mitoxantrone (FCM, n=16). Less intense treatment approaches with single-agent chemotherapies, such as F (n=37), Clb (n=21) or bendamustine (B, n=15) were chosen in 87 patients. 10 patients receiving a stem cell transplantation, experimental drugs, irradiation or splenectomy were excluded from further analyses.Treatment regimen were allocated to three different relapse treatment groups: combined chemo(immuno)therapy or single-agent antibody-treatment (e.g. A, FC, FCR, BR), single-agent chemotherapies (e.g. F, B, Clb) and chemo (immuno) therapy with anthracyclines and/or ≥3 cytotoxic agents (e.g. CHOPR, CHOP, FCM). Median treatment free survival (time from 2nd-line to 3rd-line therapy or death) was 24.5, 18.7 and 16.4 months respectively (p=0.009). These inequalities in treatment efficacy translated into a significant difference in overall survival (OS): the median OS was 78.3 and 58.2 months in patients treated with either combined chemo(immuno)therapies/single agent antibody-therapies or single-agent chemotherapies, whereas patients with combined chemo(immuno)therapies containing anthracyclines and/or ≥3 chemotherapies had the worst median OS of 42.0 months (p=0.012). A higher median age in the single-agent chemotherapy group (67 versus 60 and 61 years) might have biased the treatment selection. All other patient characteristics, such as prognostic markers (IgHV status, cytogenetics, thymidine kinase and ß2-microglobuline), response to 1st-line therapy, time between 1st- and 2nd-line therapy and ECOG status did not differ between the three groups. **Summay and Conclusions.** Patients with a relapse <24 months who received therapies that contain ≥3 cytotoxic agents and/or anthracyclines had an impaired OS. Therefore, therapies such as CHOPR, CHOP or FCM appear to be inferior in comparison to standard chemoimmunotherapies or single-agent alemtuzumab. However, the worse outcome of early relapsing patients underscores the need for alternative treatment approaches with either allogeneic stem cell transplantation or use of novel drugs.

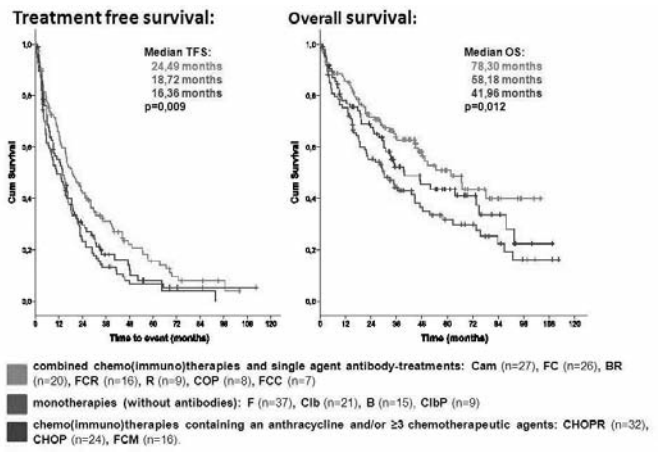

Figure 1

0148

GENETIC PROFILE OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. With the increasing complexity of recurrent mutations in chronic lymphocytic leukemia (CLL), the clinical application and translation of genetic information is challenging. As CLL patients with *TP53* abnormalities require alternative treatment approaches, there is an urgent need for rapid and reliable assessment of genetic lesions that can easily be transferred to clinical practice. **Aims.** Our aim was to apply state-of-the-art technology to genotyping of leukemia. **Methods.** We designed a sensitive and reliable multiplex PCR system to analyze recurrent mutations in CLL. Based on a test set (n=59) with parallel Sanger sequencing, we developed a 454-based next-generation sequencing (NGS) approach to rapidly profile a panel of mutations in CLL-relevant and cancer consensus genes (*BRAF, EZH2, KRAS, MYD88, NOTCH1, NRAS, PIK3CA, SF3B1, TP53*). We validated the performance of the assay in a separate validation set (n=181) and thus contribute to mapping of the genetic profile of CLL. Exons with hotspot mutations in candidate genes and *TP53* (exons 4-10) were amplified for sequencing in 2 multiplex PCRs. We aimed at a minimum coverage of 30 reads per amplicon and achieved a medium coverage of 175 reads per amplicon. **Results.** We found a total of 40 non-synonymous sequence variants in the initial patient set. The most common mutations were found in *TP53* (17/40)*, SF3B1* (11/40), and *NOTCH1* (9/40) (Figure 1). To confirm the assay performance and CLL mutation profile, we used a validation set of 181 CLL patients. The incidence of mutations and the most commonly mutated genes were similar in both data sets. All variants were verified by Sanger sequencing. In addition to known recurrent mutations in CLL, we also identified rare (but in part targetable) mutations in *BRAF* (1/40), *MYD88* (1/40), and *KRAS* (1/40) (Figure 1). Importantly, mutations in small subclones were only detectable using our newly established multiplex PCR and 454 sequencing approach. **Summary and Conclusions.** Our results provide evidence that recurrent gene mutations can be analyzed robustly with higher sensitivity than Sanger sequencing by using a NGS approach based on multiplex PCR. The in-depth analysis of the genetic profile of patients with CLL is a prerequisite for the design of genotype-specific treatment approaches. In accordance with previous studies, we show that *TP53*, *SF3B1,* and *NOTCH1* are commonly mutated in CLL.

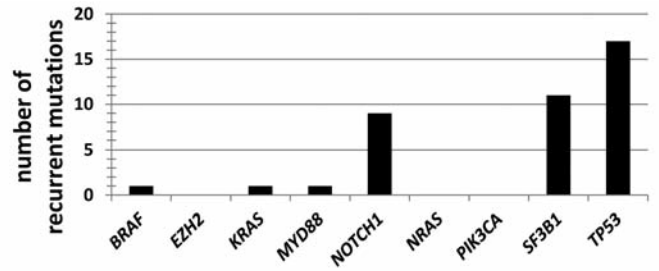

Figure 1. Distribution of recurrent mutations in CLL patients.

0149

PHASE 1B TRIAL OF AVL-292, A COVALENT INHIBITOR OF BRUTON'S TYROSINE KINASE (BTK), IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND B-NON-HODGKIN LYMPHOMA (B-NHL)

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Background. Btk supports activation, survival, and proliferation of malignant B cells in CLL and B-NHL. AVL-292 is an oral, potent (IC_{50} < 0.5nM), selective small molecule covalent inhibitor of Btk. **Aims.** To characterize the safety, dose limiting toxicity (DLT), maximum tolerated dose (MTD), pharmacokinetics (PK), pharmacodynamics (Btk occupancy), recommended phase 2 dose, and preliminary efficacy of AVL-292 in CLL and B-NHL. **Methods.** Following informed consent, patients (pts) with previously treated CLL or B-NHL received AVL-292 in escalating doses of 125, 250, 400 or 625 mg po QD per cohort using a 3+3 design in continuous 28 day cycles until progressive disease (PD) or toxicity. Plasma AVL-292 levels were assessed by LC-MS-MS. Btk occupancy by AVL-292 was assessed by covalent probe assay in blood mononuclear cells. **Results.** 16 pts have enrolled (3 each @ 125 and 250 mg, 6 @ 400 mg, and 4 @ 625 mg: 8M/8F; median age 66 years (range 45-79); median 2 prior therapies (range 1-10)) including 9 CLL (2 with del17p, 2 with del11q22, 2 with both del17p/del11q22; 2 mutated *IGHV*, 5 unmutated *IGHV*, 2 missing) and 7 B-NHL (1 diffuse large B cell lymphoma (DLBCL); 3 follicular (FL); 2 marginal zone (MZL); 1 mantle cell). Median time on treatment is 94 days, range 15-204. Thir-

teen of 16 pts continue treatment: 1 pt discontinued (dc'd) for PD after 6.75 cycles (CLL); 1 dc'd for PD after 1 cycle (DLBCL); and 1 dc'd for DLT (Gr 4 thrombocytopenia; 400 mg QD; FL). No other DLTs or grade 4 adverse events (AEs) have occurred and MTD has not been reached. Treatment emergent AEs (at least 2 subjects regardless of dose) reported as related to AVL-292 include transient diarrhea (5/16; 31.3%), and nausea and urticaria (2/16 each; 12.5%). Related Gr 3 AEs include 1 thrombocytopenia (possibly related) and 1 ANC low (probably related). Eight of 9 CLL patients have achieved best response of stable disease (SD) with median 28% decrease from baseline lymph node measurement (range 4-55% decrease; 1 pt > 50% decrease). One pt who achieved SD progressed after 6.75 cycles of AVL-292; and 1 pt is too early to evaluate for response. Six of 9 pts with CLL experienced > 10% increase in ALC over baseline in cycle 1, median increase 89.5% (range 50-212%), apparently due to re-distribution of CLL cells into the blood. Two of 7 NHL pts have SD (both MZL), 1 PD (DLBCL), 1 not evaluable due to DLT (FL), and 3 too early to evaluate. Full Btk occupancy was achieved with dose levels ≥ 250 mg. PK exposure (AUC_{last})was dose-proportional with no accumulation from Day 1 to 15. **Summary and Conclusions.** AVL-292 was well tolerated at 125, 250, and 400 mg po QD and early efficacy analysis in CLL and B-NHL shows 10/11 efficacy evaluable pts with best response of SD. Full Btk occupancy was achieved with ≥ 250 mg QD and PK was predictable with no accumulation. Additional study of 625 mg QD is ongoing. MTD cohort expansion is planned.

0150

THE INCIDENCE OF SECONDARY TUMORS IS NOT HIGHER IN PATIENTS TREATED FOR CLL COMPARED TO THE UNTREATED COHORT OF PATIENTS BUT IT IS HIGHER IN OLDER COMPARED WITH YOUNGER TREATED PATIENTS

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It is generally accepted that patients with CLL are in the higher risk of secondary tumors compared to the nonCLL population. Moreover, it has been demonstrated that some of the antileukemic treatments can be associated with secondary malignancies. There is however lack of data comparing treated and untreated populations.We have performed population based analysis of CLL patients diagnosed between 1977-2009 and recorded in the National Cancer Registry. Standard descriptive (median, percentile range) and epidemiological (crude incidence rate) statistics were used. The cumulative incidence of multiple malignancies developed over the time was estimated using standard KM estimates. Different cohorts were compared using Gray's test.Altogether 14408 pts with median age 70 years were included into the analysis. The crude incidence of tumors in this population was 2137 pts (14.9%). Out of these pts 861 (6,0%)were diagnosed before or concurrently with the CLL diagnosis. Altogether 1276 were diagnosed after the CLL diagnosis. Out of tumors after CLL dg , most frequent secondary malignancies were: lung cancer (18.8%), colorectal cancer (16.0%), prostate cancer (9.1%), renal cancer (7.4%), urine bladder cancer (5.8%), melanoma (5.1%). The cumulative incidence of secondary tumors at 5, 10 and 15 years resp. was 10, 14 and 16% resp. There was no significant difference between treated and untreated population in all time points, 9 vs 11%, 13 vs 15% and 15 vs 17% resp (ns p=0.228). We analysed two cohorts of pts - younger and older than 60 from the time of CLL dg. We have demonstrated trend (however no significant and inverse) in difference of cumulative incidence of sec. tumors between the young pts treated vs untreated in all time points (12 vs 13% at 10y, p = 0,068,) as well as in the the older population (17% vs 13% at 10y, p = 0.059). We have analysed both cohorts with the landmark analysis for treated pts from the time of first treatment and the cumulative incidence of secondary tumors in the younger cohort vs older cohort was statistically significantly different (p = 0.013), at 5, 10 and 15 y respectively it was 9% vs 14%, 13 vs 17% and 16 vs 18% resp. **Conclusions.** We have demonstrated in this population-based analysis the cumulative incidence of secondary malignancies in treated CLL patients at 15 y 17%, which is not significantly higher compared with untreated patients. The incidence is however significantly higher in older compared to younger patients. The underlying CLL seems to be more important for the onset of secondary malignancies compared to the treatment itself.

0151

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

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Background. Combination of fludarabine, cyclophosphamide and rituximab (FCR) is currently considered the treatment of choice in physically fit patients (pts) with chronic lymphocytic leukemia (CLL). However, many patients cannot tolerate this aggresive approach because of advanced age and/or serious comorbid conditions leading to unacceptable toxicity. For these patients, chlorambucil remains so far the standard of treatment. However, regimens based on low-dose fludarabine have recently demonstrated promising results in small studies. **Aims.** to assess efficacy and safety of low-dose FCR regimen used in elderly/comorbid patients with CLL/SLL: updated results are presented. **Patients and Methods.** Between March 2009 and February 2012, we treated 169 pts with active disease and efficacy/safety data are currently available on 145 pts (CLL, n=137, SLL, n=8, males, 59%, median age, 69 years [range, 58-87], median Cumulative Illness Rating Score 4.5 [range, 0-14], median creatinine clearance, 67 ml/min) by low-dose FCR at 17 centers cooperating within Czech CLL Study Group. Dose reduction of chemotherapy in comparison to regular FCR regimen was following: 50% of fludarabine dose (12 mg/m^2 i.v. or 20 mg/m^2 orally on day 1-3) and 60% of cyclophosphamide dose (150 mg/m^2 i.v./p.o. on day 1-3). Rituximab was administered in standard schedule (375mg/m^2 i.v. day 1 in 1st cycle, 500mg/m^2 i.v. day 1 from 2nd cycle). Treatment was repeated every 4 weeks. Antimicrobial prophylaxis with sulfamethoxazol/trimethoprim and aciclovir or equivalents was recommended. A total of 50% pts were untreated, remaining half had relapsed/refractory disease (median previous lines 1, range, 1-6). Advanced Rai stages (III/IV) were present in 61% pts; 39% had bulky disease. IgVH genes were unmutated in 74%; according to Doehner´s FISH hierarchical model, del 11q was present in 34% and del 17p in 6%. **Results.** Based on intention-to-treat principle, the overall response/complete response rate (including clinical CR [without bone marrow biopsy] and CR with incomplete blood count recovery) was 81/43% in first line and 69/35% in relapse. Serious (CTCAE grade III/IV) neutropenia occurred in 53%, thrombocytopenia in 12% and anemia in 12% of pts. Serious infections developed in 10% of pts. Median follow-up of living patients was 13 months (range, 2-31). Twenty-five patients have died; the most common causes of death were CLL progression and infections. Longer follow-up is necessary for PFS/OS and quality of life data. **Conclusions.** Treatment of elderly/comorbid CLL/SLL patients with low-dose FCR demonstrated promising results in first line as well as relapsed/refractory setting. Toxicity was acceptable and manageable. Recruitment in the study is ongoing and updated results will be presented. Supported by research project MZO 00179906 from Ministry of Health, Czech Republic.

0152

RELAPSES IN HAIRY CELL LEUKEMIA

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Background. Hairy cell leukemia (HCL) is an indolent lymphoproliferation effectively treated with purin analogous. However, relapses occurs and different treatment, including rituximab-containing regimens are tested. **Aims.** To estimate prognostic factors leading to relapse and survival benefit produced by addition of rituximab to purin analogous therapy. **Methods.** Between January 1995 - December 2011 196 HCL pts were treated with 2-CdA. Treatment schedules were standard. Relapse rate and its relationship to HCL form, patients clinical characteristics and results of relapse treatment were analyzed. *Results.* By February 2012r data on 165 patients was available (M:F=2,4:1, 72% classical HCL, median age 49 years, range 25-81). We detected 44 relapses in 38 (23%)

patients (M:F=2,4:1, median age 41 years (range 25-73), 71% classical HCL, 74% had 1st CR). Relapses developed within 1,5 - 12 years after 2-CdA treatment (median 3 yrs). Frequency of relapses has increased with duration of follow up from 8% in patients observed for 2 months - 6,5 years (median 3 yrs), to 61% in patients with follow up for 10 - 16 years (median 14,6 yrs). Patients with relapses (n=38) and those keeping remission (n=127) didn't differ authentically on quality of the previous remission (complete or partial), on HCL form (typical or variant), on gender (M or F). The only authentic difference was the patient's age at the moment of disease occurrence, as relapses has happened in 28 (49%) out of 57 patients younger then 45 yrs., in comparison with only 10 (9%) relapses in 108 patients older than 45 yrs. The median of disease free survival in young patients was 64 months while in older patients it was twice more and equaled 133 months However, the overall survival remained identical. What is the reason of the raised risk of relapse at the "young HCL patients"isn't clear yet. 2-CdA was administrated for relapsed HCL again, and has led to remission in 30 (91%) of 33 patients. Nevertheless the second relapse has developed later in 6 patients (5 of them young patients). Eight patients died - 3 in the 2nd CR for other then HCL reasons, 5 due HCL (2 from infections before treatment and 3 of inefficient application of 2-CdA). It has appeared that the results of the treatment of relapsed HCL depend on application rituximab if the first remission was short (remission duration less than 4 yrs). So, in 15 relapsed patients who have received only 2-CdA, the 2nd relapse developed in 5 of 7 patients (71%) with early relapse, and only in 1 of 8 patients (14%) with late relapse. However, in 11 relapsed patients who received 2-CdA plus subsequent course of rituximab the 2nd relapse hasn't developed not in 9 patients with early relapses, nor in 2 patients with late relapses with a median of observation after 2-CdA 33 months and after rituximab 12 months. *Conclusions.*We consider that 2-CdA should be followed by rituximab in HCL patients with early relapses or to prevent the relapse in HCL patients younger than 45 yrs.

0153

OTHER CANCERS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA FOLLOWED AT THE M.D. ANDERSON CANCER CENTER FOR LONGER THAN 10 YEARS

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Background. Pts with chronic lymphocytic leukemia (CLL) have an increased incidence of other cancers (OC). However, detailed description of their occurrence in pts with prolonged follow-up has not been reported. **Aims.** Report the frequency, stages, time relationship with the diagnosis of CLL, impact on survival, and types of OC occurring after treatment in pts with follow-up >10 years. **Methods.** We reviewed our database and selected untreated pts with CLL and survival >120 months from referral to MD Anderson Cancer Center. We identified pts with an OC either before or after the diagnosis of CLL.

Table 1. Distribution of OC in 694 pts with CLL and follow-up >10 years.

	ALL OC[1]				OC excluding NMSC[2]			
	Pts			Cases	Pts			Cases
	Total	OC prior/ at CLL	OC after CLL	Total	Total	OC prior/ at CLL	OC after CLL	Total
SKIN	65	23	42	78	NA	NA	NA	NA
PROSTATE	23	13	10	29	26	14	12	29
BREAST	21	10	11	24	22	10	12	24
LUNG	12	1	11	16	13	1	12	16
GI[3]	10	2	8	10	10	2	8	10
MELANOMA	6	3	3	11	10	3	7	11
H&N[4]	5	3	2	8	7	3	4	8
BLADDER	4	3	1	6	4	3	1	6
THYROID	3	2	1	3	3	2	1	3
TR MDS/AML[5]	3		3	4	4		4	4
U-V-V[6]	3	3		4	3	3		4
PNS[7]	2		2	2	2		2	2
KIDNEY	2	2		2	2	2		2
LIVER	1		1	1	1		1	1
NHL (NON-RS)[8]	1		1	3	2		2	3
PANCREAS				2				2
SARCOMA	1		1	2	1		1	2
TESTES	1	1		1	1	1		1
THYMOMA	1		1	1	1		1	1
OTHER	2		2	3	2		2	3
TOTALS (%)	166 (24)	66 (10)	100 (14)	210	114 (16)	44 (6)	70 (10)	132

[1]other cancers; [2]non-melanoma skin cancer; [3]gastrointestinal; [4]head and neck; [5]therapy-related myelodysplastic syndrome/acute myeloid leukemia; [6]uterus-vagina-vulva; [7]peripheral nervous system; [8]non-Hodgkin lymphoma (excluding Richter's syndrome)

Results. We identified 694 pts with CLL, referred between 1959 and 2001, untreated and with complete follow-up. We identified 210 individual OC in 166 pts (24%). Sixty six/166 pts (40%) had prior history of OC at the time of diagnosis of CLL or an OC diagnosed concomitantly with CLL, whereas 100 pts (60%) were diagnosed with an OC after CLL. Four hundred thirty pts (62%) required treatment, 264 (38%) were on watch-and-wait. Among treated pts 102 (24%) were diagnosed with OC, among pts on watch and wait 64 (24%) had an OC. In the treated pts, 35 (8%) had an OC detected before/concomitantly and 67 (16%) had an OC after the diagnosis of CLL. Among pts on watch and wait: 30 (11%) and 34 (13%) had an OC before/concomitantly and after CLL, respectively. The frequency, type and timing of OC are shown in Table 1. Non-melanoma skin cancers (NMSC) were excluded from subsequent analyses as all of them were localized and prognostically irrelevant. Median time to first OC was 116 (1-352) months for the entire population, 121 (4-352) in treated pts and 56 (1-273) in pts on watch and wait. In terms of survival, median overall survival (OS) is 153 (121-361) months in pts with OC, whereas it has not been reached in pts without OC with a median follow-up of 155 months (214/579, 37% dead). Moreover, median OS was 160 (121-361) months in pts who developed OC after the diagnosis of CLL and it has not been reached in pts with a history of OC prior to or at the time of CLL (median follow-up of 143 months, 19/44, 43% dead). To further identify differences between the latter two groups, we reviewed the stage of OC, excluding NMSC and hematological OC. The presence of metastases to either lymph nodes or distant organs was recorded in 6/46 pts (13%) with an OC before/concomitantly with CLL and in 17/79 pts (21%) who developed OC after CLL. **Summary and Conclusions.** In pts with CLL and >10 years of follow-up, the incidence of OC is similar in treated and watch-and-wait pts. Time to first OC is longer in pts treated for CLL compared to pts on watch-and-wait. Interestingly, the median survival of pts who developed OC after the diagnosis of CLL is 153 months, whereas it has not been reached pts without OC at a median follow-up of 155 months.

0154

CUMULATIVE ILLNESS RATING SCALE (CIRS) IS A VALUABLE TOOL TO ASSESS AND WEIGH COMORBIDITY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS FROM THE CLL8 TRIAL OF THE GERMAN CLL STUDY GROUP

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Background. A significant number of patients with chronic lymphocytic leukemia (CLL) have additional health problems, but a threshold at which comorbidity starts to influence survival and treatment toxicity is unknown. This lack of evidence makes it difficult for (i) clinicians to anticipate the impact of comorbidity on treatment outcome of individual CLL patients and (ii) scientists to define trial eligibility criteria which allow the stratification of CLL patients by medical fitness. **Aims.** To investigate whether the Cumulative Illness Rating Scale (CIRS) or other comorbidity scores are appropriate to predict mortality and treatment toxicity among the 817 subjects enrolled on the CLL8 trial of the German CLL Study Group (FC versus R-FC). **Methods.** A pretherapeutic CIRS assessment performed by the local investigators in all trial participants was used to extract information on the total score (CIRS-TOT), numbers of involved organs systems (CIRS-SYS), and organ-specific severity scores (CIRS-ORG). The Charlson Comorbidity Score (CCS) and the NCI Comorbidity Score (NCI-CS) were abstracted from the CIRS and from additional comorbidity data captured on the trial database. **Results.** The median (range of) CIRS-TOT, CCS and NCI-CS was 1 (0-8), 0 (0-4) and 1 (0-5), respectively. Of the 817 patients, 8% presented with more than 3 organ systems affected by comorbidity (CIRS-SYS >3) and 18% with at least one organ system affected by comorbidity of moderate or higher grade (CIRS-ORG =2-4). By univariate analysis, higher scores of CIRS-TOT, CIRS-SYS, CIRS-ORG, CCS, and NCI-CS all were associated with increased mortality. Predictors of reduced overall survival (OS) were CIRS-TOT >3 vs. 0-3 (HR 1.9, 95%CI 1.4-2.7, p<0.001), CIRS-SYS >3 vs. 0-3 (HR 2.5, 95%CI 1.6-3.7, p<0.001), CIRS-ORG 2-4 vs. 0-1 (HR 1.9, 95%CI 1.4-2.6, p<0.001), CCS >0 vs. 0 (HR 1.6, 95%CI 1.1-2.2, p=0.006), and NCI-CS >3 vs. 1-3 vs. 0 (HR 2.6, 95%CI 2.3-4.9, p=0.004; HR 1.5, 95%CI 1.1-2.0, p=0.006). Among these, CIRS-SYS and CIRS-ORG were the most powerful predictors of mortality in a Cox regression model. Three risk groups were identified: CIRS-SYS 0-3 and CIRS-ORG 0-1 (median OS: not reached), CIRS-SYS >3 or CIRS-ORG 2-4 (median OS: 69.7 months), CIRS-SYS >3 and CIRS-ORG 2-4 (median OS: 42.4 months). Theses CIRS parameters kept their independent prognostic value (p<0.001) in a Cox regression model including other survival factors (sex, age, performance status, Binet stage, serum factors, genomic aberrations, IGHV mutation status). Patients with CIRS-ORG 0-1 vs. 2-4 differed in their

risk of grade 3-4 toxicity (66% vs. 81%, p<0.001). **Conclusions.** This study establishes CIRS as a helpful assessment tool in CLL by identifying thresholds at which comorbidity impact on mortality and toxicity of patients treated with FC or R-FC.

0155

PHASE I FEASIBILITY TRIAL OF CYCLOPHOSPHAMIDE, ALVOCIDIB (FLAVOPIRIDOL) AND RITUXIMAB (CAR) IN PATIENTS WITH HIGH-RISK B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)/SMALL LYMPHOCYTIC LYMPHOMA (SLL)

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Background. Specific genomic [del(17p 13.1), del(11q22.3), un-mutated IgVH] and clinical [age ≥70 years] risk factors are strongly associated with poor outcomes in CLL patients. Fludarabine-based immunochemotherapy regimens, such as fludarabine, cyclophosphamide, and rituximab (FCR), have been the mainstay of therapy for CLL, despite lack of effectiveness in high-risk CLL patients and the propensity for profound immune suppression. In contrast, alvocidib, the cyclin-dependent kinase inhibitor, is effective in this high-risk group and does not promote the same cellular immune suppression typically seen with fludarabine. Additionally, cyclophosphamide has been shown to be effective specifically in patients with del(11q22.3). **Aims.** Our aim was to develop an effective regimen for nucleoside analog-naive patients, which combines the efficacy of cyclophosphamide, alvocidib, and rituximab (CAR) in a schema designed to mitigate toxicities such as tumor lysis syndrome (TLS) and cytokine release syndrome (CRS). Our primary goals were to determine the tolerability and feasibility of frontline administration of combination CAR therapy to patients with high-risk CLL. **Methods.** We conducted a phase I trial of CAR in symptomatic CLL/SLL patients with high genomic or clinical risk disease. All patients had ≤1 prior therapy, ECOG performance status of 0-2, and adequate organ function. Patients in all cohorts received rituximab 100mg IV day 1 (of 21) of cycle 1 and 375 mg/m^2 IV on day 2 of cycle 1 and day 1 of cycles 2-6. Patients received either 300 mg/m^2 (cohorts 1 and 2) or 375 mg/m^2 (cohort 3) of IV cyclophosphamide on days 1-3. Patients received 30 mg/m^2 IV alvocidib over 30 minutes, followed by 30 mg/m^2 (cohort 1) or 50 mg/m^2 (cohorts 2 and 3) IV alvocidib over 4 hours day 8 of cycle 1 and days 1 and 8 of cycles 2-6. Standard 3x3 design was utilized with nine patients completing therapy, three in each cohort. **Results.** Median age was 55 years (range 43-77 years) and 7 were male. Patients had the following high risk features: del(17p 13.1) (*n*=3), del(11q22.3) (*n*=4), un-mutated IgVH (*n*=7), and age >70 years (*n*=2). All patients completed 6 planned cycles with exception of one patient in cohort 2, who stopped therapy after cycle 4 due to lack of response. Dose limiting toxicity was not reached. Toxicities observed were typical of those reported with the three individual drugs and were manageable. Importantly, there were no instances of TLS or significant CRS.All 9 patients were evaluable for response by 2008 IWCLL criteria. Three patients achieved CR, 4 achieved PR, 1 had stable disease, and 1 had progressive disease (PD). The patient with PD was 77 years old and had del(17p13.1) and un-mutated IgVH cytogenetics. **Conclusions.** Combined therapy with CAR is well tolerated in patients with genomic and clinical high-risk CLL. The known severe side effects of TLS and CRS were not observed with the novel timing of alvocidib in this regimen. This combination could offer advantages over standard frontline therapies for CLL patients in this high-risk group. Further study of this regimen is warranted in the setting of a phase II clinical trial.

0156

PROGNOSTIC RELEVANCE OF CD49D EXPRESSION ON B LEUKEMIC CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA. META-ANALYSIS OF PUBLISHED AND UNPUBLISHED INDIVIDUAL DATA FROM 3146 PATIENTS.

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Background. A number of investigators have provided data supporting the value of CD49d expression on B leukemic cells in chronic lymphocytic leukemia (CLL) as independent prognostic variable. These studies used a variety of clinical end points including overall survival (OS), time to treatment (TTT) or treatment free survival (TFS). The factors included in multivariate analyses of these studies also differed. Unresolved issues regarding the prognostic value of CD49d assessment in CLL included the choice of the optimal cut-off to define positivity and CD49d prognostic value in patients subsets defined by other standard prognostic factors. **Aim.** To perform meta-analysis using individual patient level data from studies of CD49d expression to evaluate its ability to predict OS (primary end-point) and TTT/TFS (secondary end-point). **Methods.** Studies published by 30 April 2011, reporting an association of high CD49d expression on B CLL cells, measured by flow cytometry, and end-points were identified by Medline search. Additionally, we performed a manual review of abstracts presented at the congress of the American Society of Hematology from 2006 to 2010. CD49d was used as a categorical variable, as coded in original studies.*Results.* We identified 6 published studies and one abstract for inclusion. All authors agreed to provide individual data on the patients in these publications as well as unpublished data on additional patients to be used for replication analysis. Four authors also submitted updated follow-up data. We collected the following variables: date of diagnosis, OS, TTT/TFS, CD49d, CD38, ZAP-70, immunoglobulin mutational (IGHV) status, del17p and del11q chromosomal aberrations, age, stage, ALC, and β2 microglobulin concentration. Data from 3146 individual patients was available with 261 subsequently excluded due to missing end point and/or CD49d data. Of 2771 patients with valid data, 1405 (51%) were included in the previous publications and 1366 (49%) were unpublished. Before starting analyses a decision was made to perform meta-analysis on published data and use data from previously unpublished data as a validation cohort. Pooled CD49d hazard ratio for OS was 2.62 (1.88-3.64). In bivariate analysis, the prognostic value of CD49d was confirmed and of comparable value in patients subsets defined by CD38 and ZAP-70 expression, IGHV status, unfavorable chromosomal aberrations. Finally, we performed a multivariate analysis including CD49d, CD38, ZAP-70, IGHV status, del17p and del11q. CD49d was significantly associated with shorter overall survival in this adjusted model, with a hazard ratio of 2.47(1.17-5.21). Inspection of Martingale residuals plots of CD49d in each study failed to show a recognizable cut-point. Accordingly, rather than using a data driven approach, these analyses suggest choosing a cut-point on pure distributional criteria or by a data independent approach. Analysis using such a strategy is underway with plans for validation in the cohort of 1366 unpublished patients. **Conclusions.** Preliminary results of a meta-analysis using individual patient data from >1400 patients confirm the association of high CD49d expression with short OS independent of other prognostic parameters. These findings may have implication for patient's stratification in future prospective studies and potential therapeutic efforts to target CD49d or CD49d signaling.

0157

APPLICATION AND FEASIBILITY OF ALLOGENEIC STEM CELL TRANSPLANTATION IN T-PROLYMPHOCYTIC LEUKEMIA: A SINGLE INSTITUTION EXPERIENCE OF 27 PATIENTS

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Background. T- prolymphocytic leukemia (T-PLL) is an aggressive T cell neoplasm associated with a poor response to chemotherapy and a median survival of < 1 year. Therapy with alemtuzumab has improved overall response (51-76%) and complete remission rates (40-60%); however the median survival continues to remain short. Finally, recent data has pointed towards potential benefit in patients with T-PLL from allogeneic hematopoietic stem cell transplantation (HSCT) within a short interval from diagnosis using a preparative regimen including total body irradiation (TBI). **Aims.** We undertook this study to estimate the feasibility and outcomes related to chemo-immunotherapy and HSCT in patients with T-PLL. **Methods.** 27 consecutive patients with T-PLL were seen at the Mayo Clinic from 1997 through 2011. All patients underwent peripheral blood flow cytometry, bone marrow examination and cytogenetic evaluation at diagnosis. T-PLL cells were characterized by a CD2+, CD3+, CD5+, CD7+ phenotype positive for CD4 and/or CD8. Cytogenetic studies included FISH analysis for *TCL1* at 14q32, deletion 11q23 (*ATM*), trisomy 8 and deletion 17p (*TP53*). All clinical and demographic data were retrospectively abstracted. Cox proportional regression method was used for multivariable analysis. **Results.** Of the 27 study patients, 14 (51%) were female and median age was 65 years (range, 40-86). The median (range) hemoglobin was 13.2 gm/dl (6-15.9), white cell count 74 x10^9/L (7-551), absolute neutrophil count [ANC] 6 x10^9/L (1-21), absolute lymphocyte count [ALC] 70 x10^9/L (2.7-540) and platelet count 153 x10^9/L (29-299). On FISH analysis 15 (56%) had abnormalities of 14q32 and 3 of these had +8 (11%). At last follow up 22 (81%) deaths were recorded. Two patients (7%) have an indolent disease that has thus far not necessitated ther-

apy. Seventeen patients were treated with alemtuzumab based regimens, while 8 received purine nucleoside analogues (cladribine/fludarabine/pentostatin). The median survival in the patients receiving alemtuzumab was 7 months compared to 4 months (p=0.5). Eight patients (30%) were >70 years of age and hence not eligible for HSCT. Of the 19 transplant eligible patients, 14 were unable to proceed to transplantation due to either progressive disease or lack of a suitable donor source [3 (22%)]. Thus only 3 (11%) transplant eligible patients were able to undergo an allogeneic HSCT (3 myeloablative matched unrelated donor HSCT). Two of the 3 transplanted patients died within 9 months from complications related to disease relapse. In a univariate survival analysis, high white count at presentation, high ALC, low ANC and cytogenetic abnormalities in addition to those involving 14q32 were predictive of a shorter survival, whereas in a multivariable model only the low ANC retained significance [p=0.04, HR 0.123-2.65]. **Summary and Conclusions.** Although transplant is considered the only therapy that offers long-term disease control for patients with T-PLL, only ~10% of patients in this series were able to proceed with allogeneic HSCT because of i) inadequate disease control with induction, ii) advanced age and iii) limited donor options. Better induction chemoimmunotherapy and the use of alternative donor sources (haploidentical/umbilical cord blood) need further exploration.

0158

COMPARISON BETWEEN THE INTERNATIONAL STANDARDISED APPROACH AND A SINGLE TUBE TEN COLOUR FLOW CYTOMETRIC ASSAY FOR DETECTION OF MINIMAL RESIDUAL DISEASE IN CLL

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Background. The eradication of minimal residual disease (MRD) in CLL predicts for improved outcome. Approaches to CLL MRD detection vary in sensitivity and cost. Historically, CD5/CD19 co-expression with demonstration of clonality has been the principal method of MRD evaluation in CLL. However this approach is limited by the identification of normal hemopoietic cells and the inability to demonstrate light chain restriction with very low B cell numbers. More recently, an international standardised approach (ISA) to the assessment of MRD in CLL that utilises 10 monoclonal antibodies in a 4 tube test system and permits a sensitivity of 0.01% (Rawstron et al, Leukemia 2007) was published. **Aims.** To develop a single tube 10 colour flow cytometric assay based on the ISA methodology to be used in a centralised flow cytometry laboratory providing results for a multicenter clinical trial of lenalidomide treatment for MRD following FCR chemotherapy. The method was designed to minimise the requirement for cell numbers in patients with relative leucopenia following chemotherapy.Method: Peripheral blood (n=51) and bone marrow (n=20) was collected from patients at various time points post treatment for CLL (including bone marrow from 3 patients day 30 post-allogeneic stem cell transplant). Immunophenotyping was performed using a Gallios flow cytometer (Beckman Coulter). Monoclonal antibodies were used either according to the ISA (antibody/fluorochrome combination as per Rawstron et al) or in a single tube incorporating all of the following monoclonal antibodies: CD3 ECD, CD5 PercP5.5, CD19 eFluor, CD20 APC CY7, CD81 FITC, CD22 PE, CD43 PECY7, CD79b APC, CD38 A700, CD45 KO. A whole blood or bone marrow lyse method was used and analysis was performed according to the published ISA methodology or for the single tube using templates based on the ISA approach. Results from the two methods were compared. **Results.** Levels of residual disease in the 71 samples analysed varied from <0.01 to 22%. Twenty-four samples showed residual disease of <1%. Analysing all samples showed an excellent correlation between the two methods slope = 0.989, intercept =0.1 and R(2)=0.992. There was also excellent correlation for disease levels below 1.0%, (median 0.03 range <0.01-0.63%, n=24) slope = 1.15, intercept =0.007 and R(2)=0.994. Bland Altman analysis showed a mean of 0.008 +/- 0.058 (2SD) for values below 1%. **Conclusions.** The single tube ten colour flow cytometric assay for detection of MRD in CLL gives equivalent results to the ISA. There is a potential for improved sensitivity resulting from the removal of CD19+/CD3+ contaminating events and by increasing the total number of events acquired since there is no need to divide the sample into multiple tubes, particularly post-treatment when cell numbers are frequently limiting. The single tube assay is also simple, rapid and cost effective.

0159

A HIGH NUMBER OF LOSSES IN 11Q CHROMOSOME IS ASSOCIATED WITH A WORSE SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. PRELIMINARY RESULTS OF DATABASE OF CLL OF SPANISH GROUP OF CYTOGENETICS

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Background. Cytogenetic abnormalities in CLL patients define groups of patients with different prognosis. Recently, three independent groups have reported that a high number of losses in 13q14 is related with a worse prognosis as well as it can be observed in patients with CLL and 17p deletion associated or not with *TP53* mutation. CLL patients with 11q deletion have a bad prognosis, although with a variable outcome. **Aims.** To analyze in a Spanish multicentric study if the number of losses in 11q chromosome in patients diagnosed with CLL can influence in overall survival (OS) and time to the first therapy (TFT). **Methods.** A total of 1.303 patients registered in DataBase of CLL of Spanish Group of Cytogenetics (GCECGH) and Spanish Group of CLL (GELLC) were included. All the clinical data as well as FISH information (13q, 12, 11q, 14q and 17p probes) and molecular studies were carried out. **Results.** One hundred and thirteen patients (8.67%) had 11q deletion, although the analysis was limited to 66 cases (52 men) due to lack of clinical data or inappropriate follow-up. Median OS of patients with 11q- was 85 months (CI 95%, 59-109) and TFT was 33 months (CI 95%, 18-48). At the moment of analysis, 30% (20/66) of patients had died and 64% (42/66) had progressed. In 28 out of 66 patients (42%) loss of 11q was the sole cytogenetic aberration at diagnosis. Most of patients (62%) were in clinical stage A of Binet. Interestingly, in the subgroup of patients with ³ 40 % of cells with loss of 11q (54 cases, 81%), the OS was 84 months (CI 95%, 67m-101m), while in the group with < 40 % of losses in 11q, the OS has not been reached (CI 95%, 99m-132m) (P= 0.045). In the univariate analysis for OS, a high serum LDH level (P= 0.026), a high serum $\beta_2$microglobulin level (P= 0.022) and unmutated *IGVH* pattern (P= 0.045) were associated with a worse survival, and a trend in advanced stages of Binet (P= 0.10) and lymphocyte blood count >30 x 10^9/L (P = 0.11) was observed. In the multivariate analysis for OS, the only significant variable included in the final model was a high serum LDH level (P= 0.043). Regarding the time to first therapy, in the group with ≥40% of losses in 11q the median TFT was 20 months (CI 95%, 1-41) while in the group of patients with <40% of 11q- cells it was 52 months (CI 95%, 32-72) (P = 0.087). In the TFT univariate analysis differences were observed in cases of high serum LDH (P = 0.003) and lymphocyte blood count >30 x 10^9/L (P = 0.033). **Conclusions.** In our series, the incidence of 11q deletion is less than described in the literature. Our results suggest that in patients with CLL, a high number of losses in 11q is inversely related to OS and shows a trend to a short TFT.

0160

CISPLATIN CONTAINING SALVAGE TREATMENT OF P53 DYSFUNCTIONAL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH BULKY DISEASE IS HIGHLY EFFECTIVE AND RESULTS IN TAP73 MEDIATED APOPTOSIS

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Background. In CLL, loss of function of p53 is highly associated with fludarabine-refractory disease and poor outcome. We recently reported activity of a cisplatin containing regimen (R-DHAP; rituximab, dexamethasone, cytarabine and cisplatin) in CLL in a retrospective analysis[1]. In solid tumors the activity of platinum-based compounds such as cisplatin was found to be independent of p53 function and mediated by the p53 family member TAp73. **Aims.** We further analyzed efficacy of a platinum-based regimen in fludarabine-refractory CLL patients and studied the role of TAp73 in cisplatin induced apoptosis. **Methods.** We performed an interim-analysis of a nation-wide multi-center phase 2 clinical trial in which fludarabine-refractory CLL patients with progressive disease receive 3 cycles of R-DHAP prior to allogeneic stem cell transplantation. Next, the mechanism of action of cisplatin was studied in patient derived p53 dysfunctional CLL cells and in the pro-lymphocytic cell line MEC1. Informed consent was obtained in accordance with the Declaration of Helsinki. **Results.** A response (according to IWCLL 2008 criteria) was obtained in 9 of 15 patients (60%) treated with R-DHAP; 4 of 8 patients (50%) with bulky disease responded. *In vitro* treatment of quiescent peripheral blood derived CLL cells with cisplatin did not result in cell death. However, cisplatin did induce apoptosis in p53 dysfunctional CLL cells which were brought to proliferate by combined stimulation with CD40-ligand and CpG (as a model for the lymph node (LN) environment).The prolymphocytic cell line MEC1 is p53 dysfunctional and has an activated NF-κB signature and can hence function as a model for p53 dysfunctional CLL cells in the LN environment. In MEC1 cells, cisplatin treatment resulted in cell cycle arrest, apoptosis and also increased sensitivity to FAS (CD95)- and fludarabine-induced cell death. At the molecular level, treatment with cisplatin induced c-Abl dependent protein expression of TAp73 and its downstream targets p21, Bid, Puma and CD95. TAp73 RNA interference markedly decreased sensitivity to cisplatin, supporting a role for TAp73 in apoptosis regulation in these p53 dysfunctional cells.Clinical relevance of these findings is supported by the observation of increased expression levels of TAp73 in lymph node derived CLL cells as compared to peripheral blood derived cells. Moreover, expression of TAp73 was observed in primary patient derived CLL cells upon stimulation with CD40-ligand (and CpG), suggesting that TAp73 induction in primary CLL cells is restricted to the activated compartment. Lastly, in p53 dysfunctional CLL cells isolated 24 and 48 hours after *in vivo* treatment with cisplatin, a clear induction of TAp73 was observed. This correlated with a rapid decrease in peripheral blood leukocyte count and lymph node size. **Conclusions.** These data indicate that platinum-based compounds are active in fludarabine-refractory (p53 dysfunctional) CLL. The activity of cisplatin in p53 dysfunctional CLL may at least in part be mediated by induction of TAp73; especially in the context of proliferation as occurs in the LN environment.

Reference

1. Tonino SH, van Gelder M, Eldering E et al. R-DHAP is effective in fludarabine-refractory chronic lymphocytic leukemia. Leukemia 2010;24:652-654.

0161

TREATMENT OF REFRACTORY HAIRY-CELL LEUKEMIA BY BRAF INHIBITION

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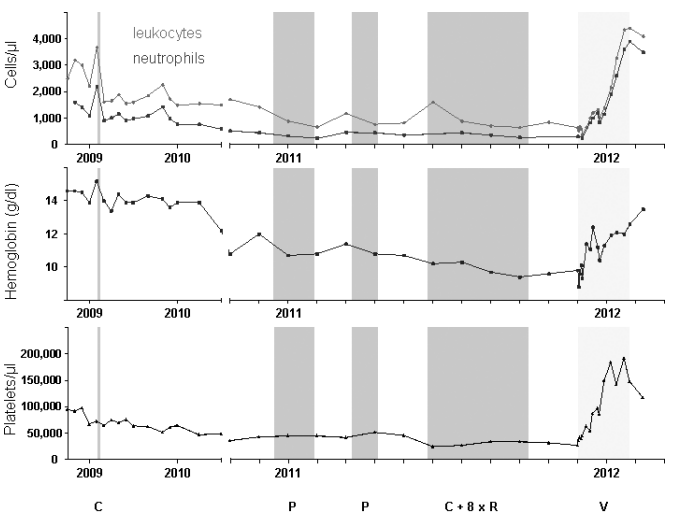
Background. Targeted intervention against driver mutations is about to open a new era in cancer treatment. An activating mutation of the BRAF serine/threonine protein kinase, BRAF V600E, occurs in a significant proportion of malignant melanomas, colorectal, thyroid, and other cancers. In hairy-cell leukemia (HCL), BRAF V600E is nearly always present, suggesting disease-specific oncogene dependence. **Methods.** Here, we present a patient with chemotherapy-refractory HCL who was treated with a short course of vemurafenib, a specific inhibitor of mutant BRAF. The activating *BRAF* V600E mutation in the HCL clone of this patient was demonstrated by DNA sequencing and a mutation-specific antibody. **Results.** Before vemurafenib treatment, no objective response could be achieved by three lines of purine analogue-based treatment regimens (cladribine, pentostatin and rituximab/cladribine). At the time of treatment initiation with vemurafenib, the patient had a BM infiltration of 70%, massive splenomegaly (24.8 x 8.3 cm), severe pancytopenia (leukocytes, 680/μL; hemoglobin, 10 g/dL; platelets, 36,000/μl) and a serum CD25 (soluble interleukin 2 receptor) level of 24,800 U/ml (normal rage, <900 U/mL). Treatment was started with 240 mg twice daily after a single loading dose of 960 mg and slowly escalated to 1,920 mg/d, the dose established in treatment of melanoma. Within two days of treatment with 480 mg/d, the spleen softened and decreased in size. On day six and 17 of vemurafenib treatment, the spleen size had shrunk to 18.8x6.9 cm and 14x5 cm, respectively. Platelets, hemoglobin and neutrophils normalized rapidly (neutrophils ≥1,500/μL on day 36; hemoglobin ≥12 g/dL on day 43; platelets ≥100,000/μL on day 28; Figure 1. Serum CD25, a reliable marker of HCL load, fell rapidly below 900 U/l by day 28. There was no clinical evidence of a tumor lysis syndrome associated with the rapid response to vemurafenib treatment. On day 43 of treatment, complete remission (CR) criteria were achieved. Treatment was terminated on day 56. At this point, there was no evidence of disease persistence based on immunophenotyping of the peripheral blood and BM histological findings, but a small HCL clone (<0.1%) was detected by flow cytometry of the BM sample. Final BM evaluation on day 70 confirmed CR while peripheral counts remained stable. **Conclusions.** The striking therapeutic activity of vemurafenib in our patient provides the first functional evidence to substantiate *BRAF* V600E as a driver oncogene *in vivo* and to validate mutant BRAF as a therapeutic target in HCL, thereby adding to the recent success of molecular mechanism-based therapies in other kinase-driven malignancies. Given the rarity of HCL, introducing new therapeutic modalities may be challenging. Future studies are needed that compare short-term inhibition of BRAF with low doses of vemurafenib (or alternative BRAF inhibitors) with standard therapy.



(C: cladribine, P: pentostatin, R:rituximab). With start of vemurafenib (V) treatment, the peripheral blood counts normalized rapidly to normal levels.

Figure 1. Low-dose Vemurafenib (V) leads to Normalization of Blood Counts in Hairy-cell Leukemia.

0162

POOR PROGNOSIS OF HODGKIN VARIANT OF RICHTER'S TRANSFORMATION IN CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH CLADRIBINE

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It is generally believed that chronic lymphocytic leukemia (CLL) transformation to Hodgkin lymphoma (Hodgkin variant of Richter's transformation, Hodgkin-RT) has superior outcome than more common Richter's transformation to diffuse large B-cell lymphoma (DLBCL-RT). However, recent observation suggested that Hodgkin-RT in patients in whom CLL had been treated with fludarabine have very poor survival (Bockorny et al, 2012). In order to evaluate the clinical course of Hodgkin-RT in patients treated previously with cladribine we performed a single-center retrospective analysis to compare Hodgkin-RT incidence, characteristics and outcome with those of DLBCL-RT. We searched electronic databases of the Departments of Hematology and Pathology of the Medical University of Lodz to identify patients who developed biopsy- or fine-needle aspiration-proven RT between January 2000 and November 2011. Hospital records and ambulatory charts were reviewed to determine clinical, laboratory and pathologic features, treatment and outcome of CLL and RT. Hodgkin-RT and DLBCL-RT characteristics were compared by Fisher exact test or Mann-Whitney test. Survival after transformation was plotted using the Kaplan-Meier method and comparisons made by the log-rank test. **Results.** Among 786 patients with CLL admitted to our center since January 2000, 40 (5.1%) patients developed RT including 33 (4.2%) DLBCL-RT, six (0.8%) Hodgkin-RT and one plasmablastic lymphoma. There were 23 male and 17 female patients with a median age of 67.2 years (range 35.8-84.9). RT was diagnosed after a median of 2.6 years (range 0-12.3) from CLL diagnosis. Median survival after transformation was 1.1 years, 95% confidence interval (95%CI) 0.4-1.8 years. Of the patients with Hodgkin-RT, there were five men and one woman with a median age of 70.6 years (range 55.8-76.3). All patients received cladribine-based regimens before transformation. Treatment in Hodgkin-RT was mostly HL-type therapy including ABVD, COPP an COP. Two partial remissions were achieved, while four patients did not respond to chemotherapy. During the follow-up period five patients died of disease progression at 0.5, 2, 4 and 11 months from transformation, whilst one patient remained alive after 7 months of observation. Most of DLBCL-RT patients received rituximab-based immunochemotherapy. The comparison of patients with DLBCL-RT and Hodgkin RT indicated similar presenting characteristics and time to transformation in both types of RT, Interestingly, we found significantly shorter survival after transformation in Hodgkin-RT compared to DLBCL-RT (p=0.019) (Figure 1). In conclusion, the results of this study indicate that Hodgkin-RT in cladribine-treated CLL patients is characterized by very short survival, even compared to DLBCL-RT. This may be due to common choice of alkylator-based HL-type therapy that is likely suboptimal for Hodgkin-RT in patients exposed to purine analogs. We propose that more intensive treatment, such as immunochemotherapy with rituximab or alemtuzumab should be considered for Hodgkin-RT in CLL patients who had been previously treated with purine analogs.

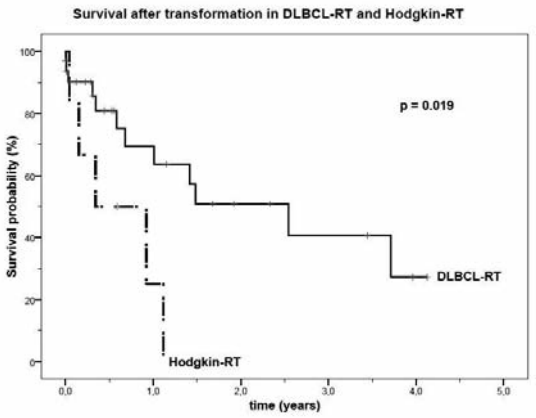

Figure 1.

0163

ASSESSMENT OF P53 FUNCTIONALITY IN CHRONIC LYMPHOCYTIC LEUKEMIA BY DIFFERENT ASSAYS; AN ERIC-WIDE APPROACH

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Introduction. The DNA damage response axis plays a crucial role in chemoresistance in CLL, as indicated by the prognostic impact of deletions of 17p (locus of *TP53*) and 11q (locus of *ATM*). These deletions coincide with mutations in the remaining allele, although frequency, especially for *ATM* varies. Functional read-outs of the p53 axis might add clinical relevant information on the actual DNA damage response. Currently, different p53 function analyses are being developed. These assays are either based on measurement of (i) RNA expression levels of a single gene (RT-PCR*p21*) or gene sets (RT-MLPA), or protein expression levels (FACSp53-p21) after DNA damage by irradiation or etoposide/nutlin exposition or (ii) gene expression levels at base-line (RT-PCR *miR34a*). To what extent these different assays correlate is currently unknown. **Aims.** Detailed side-by-side analysis of available p53 functional assays in well characterized CLL samples **Methods.** Freshly frozen PBMC's of 15 different CLL samples (CD19/CD5>90%) were exchanged between 5 research groups that developed the following p53 functional assays: RT-PCR*p21* (Ulm), RT-MLPA (*bax*, *puma*, *p21* and *CD95*; Amsterdam), FACSp53-p21 (Paris) and RT-PCR *miR34a* (Salzburg/Brno). FISH-analysis (11q, 17p, 13q and 12) was performed on all samples. In addition mutations in *TP53* were determined by FASAY, DHPLC and Sanger sequencing and *ATM* mutations were assessed by Sanger sequencing. **Results.** RNA expression levels showed significant correlation (p-value < 0,01) between the different P53 functional assays with high correlation coefficients (range: 0,7-0,94). Based on combination of FISH-analysis, FASAY and Sanger sequencing the investigated CLL samples could be distinguished into 6 different categories; 1. 17p-with *TP53* mutation (n=5), 2. Sole *TP53* mutation (n=1), 3. 11q- with *ATM* mutation (n=1), 4. 11q- in absence of *TP53/ATM* mutation (n=4), 5. 17p- with both *TP53* mutation and *ATM* mutation (n=1) and 6. No 17p- and 11q- in absence of *TP53/ATM* mutation (n=3). All p53 functional assays showed absent to minimal induction of expression of respective target genes in samples with a cytogenetic abnormality combined with a *TP53/ATM* mutation (category 1, 3, 5). In contrast, samples without a cytogenetic abnormality in absence of *TP53/ATM* mutations (category 6) showed a marked increase in expression of respective target genes in all the assays. The patient with a sole *TP53* mutation (category 2) showed a normal DNA damage response in all assays except for FACSp53-p21. Samples with an 11q-in absence of *TP53/ATM* mutation (category 4) showed high inter-assay variation. The two assays with predefined cut-off values (RT-MLPA and FACSp53-p21) assessed 13 samples equally (9 p53 dysfunctional and 4 p53 functional), except for 2 patients (category 2, 3 respectively). Reproducibility of these assays is currently being determined. **Discussion.** For the first time a comparative side-by-side analysis of different available p53 functional assays was performed, which demonstrated strong correlations between the different assays. All assays could detect samples with expected disturbed and normal DNA-damage responses. To what extent these p53 functional assays could be of clinical relevance especially with respect to chemo-responsiveness, should be further studied in prospective studies.

Chronic myeloid leukemia - Biology

0164

CBL DOWNREGULATION PLAYS A CRITICAL ROLE IN THE INCREASE OF AXL AND LYN TYROSINE KINASE LEVELS MEDIATING RESISTANCE OF CHRONIC MYELOID LEUKEMIA CELLS TO NILOTINIB

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The second generation of Abl tyrosine kinase inhibitor (TKI) nilotinib has been developed to overcome the resistance to imatinib, which was up to now the gold standard treatment of chronic myeloid leukemia (CML). Nilotinib has been approved recently as the front line treatment of CML but in rare situations, primary and secondary resistances are observed. We generated nilotinib-resistant K562 CML cells and characterized the mechanism responsible for nilotinib resistance in these cells : overexpression and increased activity of the Src-like tyrosine kinase Lyn (Mahon et al, 2008). A quantitative phosphoproteomic analysis performed by "Stable isotope labeling with amino acids in cell culture"(SILAC) further showed that this activity of Lyn is mediated by overexpression of the tyrosine kinase Axl and the hyper activation of the Spleen tyrosine kinase Syk (Gioia et al, 2011). The step by step inhibition of these tyrosine kinases confirmed the relevance of each of them in the resistance to nilotinib. Similar mechanisms were detected in primary cells from nilotinib-resistant CML patients. The mechanism of deregulation of Lyn is still currently unknown. Down-regulation of Axl by a specific shRNA strategy in nilotinib-resistant K562 cells (K562-rn), which overexpressed Axl, abolishes resistance that correlates with a decrease of Lyn overexpression and an increase of Cbl expression. Interestingly, from the SILAC analysis we observed a down-regulation of several signaling proteins including the ubiquitin E3-ligase c-Cbl. Since Cbl targets several tyrosine kinases for lysosomal recycling or proteosomal degradation, we addressed whether Cbl inhibition plays a role in Lyn and Axl protein accumulation. Cbl depletion in K562 cells by a specific shRNA strategy induced a large increase of Axl and Lyn protein levels. In addition, the depletion of Cbl also induced an emerging resistance to nilotinib as assessed in viability and apoptosis assays. Conversely, the overexpression of Cbl in nilotinib-resistant K562 cells (K562-rn) dramatically reduced Axl and Lyn levels and these effects were dependent upon an intact E3-ligase activity of Cbl. Last, Cbl expression re-sensitized K562-rn cells to nilotinib. Altogether, these results indicate that Cbl down-regulation plays a critical role in the increase of Axl and Lyn levels that control resistance to nilotinib in CML cells.

0165

DASATINIB MAY OVERCOME THE NEGATIVE PROGNOSTIC IMPACT OF KIR2DS1 IN NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Natural killer (NK) cells are an important component of the allo-immune effect following allogeneic stem cell transplantation and have been shown to exert direct cytotoxicity against primitive quiescent CD34+ Philadelphia positive cells *in vitro*. The balance between signals received from inhibitory and activating cell surface receptors determines the cytotoxic activity of NK cells. These receptors include the killer immunoglobulin-like receptors (KIR).We recently reported on the relationship between KIR genotype and outcome in a large homogeneous cohort of newly diagnosed patients with CML in chronic phase (CP) treated with imatinib (Marin, Leukemia 2011). We showed that patients carrying the activating KIR gene *KIR2DS1* have a significantly lower probability of achieving CCyR, and lower 2-year probabilities of progression-free survival (PFS) and overall survival (OS) compared with those who are *KIR2DS1* negative. This effect was independent of Sokal risk score and was validated in a second independent large cohort of 174 patients with CML-CP treated with first-line imatinib in the multi-center UK SPIRIT 1 trial. The impact of the *KIR2DS1* genotype on the probability to achieve CCyR was even greater when the expression of this gene was considered together with the presence or absence of the ligand for the corresponding inhibitory KIR receptor (KIR2DL1), suggesting that in the presence of the HLA ligand, the corresponding inhibitory KIR may neutralize the effect of the activating KIR. We concluded that *KIR2DS1* may predict response to imatinib and identify patients at greater risk of treatment failure.In this study, we aimed to investigate whether *KIR2DS1* could also predict response to treatment with dasatinib. Dasatinib, a second generation TKI with potent BCR-ABL inhibitor activity, induces deeper and faster clinical responses than imatinib. Dasatinib also inhibits a wide variety of other kinases such as SRC and TEC, known to be key regulators of immune response.We studied 130 patients with CML in CP treated with first-line dasatinib on the UK multi-center trial (SPIRIT 2) for whom genomic DNA samples were available for analysis. All patients gave written informed consent for the use of their data for this analysis. The median follow-up was 18 months and 25% of the patients have been followed for at least 24 months. During follow-up 122 (93.8%) patients achieved CCyR and 94 (72.3%) major molecular response (MMR). We found no significant impact of KIR genotype on outcome. Specifically, the probabilities of CCyR, MMR and CMR for patients who were *KIR2DS1* allele positive was not statistically different, although marginally better compared to those patients who were negative for this gene, namely RR= 1.398 (p=0.09), RR= 1.003 (p=0.98) and RR= 1.526 p=0.64 respectively. These data indicate that dasatinib may overcome the negative prognostic impact of *KIR2DS1* on CCyR in newly diagnosed patients with CML treated with imatinib. Longer follow-up is needed to assess whether dasatinib also overcomes the negative impact of *KIR2DS1* genotype on PFS and OS.Functional and phenotypic studies to determine the differential impact of imatinib and dasatinib on KIR2DS1 expressing NK subsets are currently under way.

0166

A DNA METHYLATION CLASSIFIER PREDICTS RESPONSE TO IMATINIB IN PRE-TREATMENT CML SAMPLES

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Background. Despite the consistent nature of the BCR-ABL1 fusion gene identified at diagnosis, CML is clinically heterogeneous as exemplified by the predictive value of Sokal scores and the variable response to tyrosine kinase inhibitors (TKI). We speculated that aberrant epigenetic programming and in particular differential DNA methylation might underlie the variations in outcome and variable responses to imatinib. **Methods.** $CD34^+$ cells were purified from the blood of 46 patients with CML-CP at diagnosis. Patients were treated with imatinib (400 mg/day) and classified as responders (n=29) if they achieved durable complete cytogenetic response (CCyR). Responders were subjected to leukapheresis following G-CSF mobilization. Non-responders (n=17) never attained major cytogenetic response (MCyR) (n=13) or lost a previously attained CCyR despite continuing imatinib (n=4). Using the Illumina Infinium HumanMethylation450 BeadChip to scrutinize 429,231 CpG dinucleotides within the genome, the pre-treatment genome-wide methylation profiles of the responders were compared with those of the non-responders as well as with the corresponding paired CCyR sample. Analogous methylation signatures were obtained from $CD34^+$ cells collected from healthy donors (in excess of requirement) treated with G-GSF to yield cells destined for allogeneic stem cell transplantation. Using Random Forest (RF), an ensemble classifier (supervised learning algorithm), a model was developed to define the CML-CP methylome and to separate imatinib responders from non-responders based on their genome-wide DNA methylation profile. With a permutation test it was then possible to select individual probes that separated these two groups ($p<0.01$). **Results.** Unsupervised hierarchical clustering of all samples using Ward's method showed that genome-wide DNA methylation analysis clearly distinguishes between CML-CP, CML-CCyR and normal $CD34^+$ cells. Two clusters, one derived from diagnostic CML samples and the other from the controls and CCyR samples (i.e. minimal or no leukemia) were identified. In the latter cluster CCyR samples were distinct but epigenetically much closer to normal $CD34^+$ cells than CML-CP samples. 534 different probes separated responders from non-responders. Using these 534 probes a 'leave-one-out' cross-validation procedure was repeated 100 times to estimate their prediction accuracy; their use correctly predicted all responders. **Conclusions.** The work so far has identified consistent differences in genome-wide methylation patterns between patients with untreated disease versus normal controls indicating a possible common epigenetic pathway in the leukaemic transformation to leukaemia. The study has also defined a 534-probe DNA methylation classifier, which predicts overall response to imatinib in CML patients. Validation with a second independent patient cohort is currently in progress.

0167

ABLATION OF ATG7 AUTOPHAGY GENE EXPRESSION AUGMENTS TKI EFFECTS AND ENHANCES CML CELL DEATH

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Background. Imatinib, a BCR-ABL tyrosine kinase inhibitor (TKI), has revolutionized treatment of chronic myeloid leukaemia (CML). Despite imatinib's auspicious effects, it fails to cure the disease due to CML stem cells' refractoriness and development of resistance (e.g. BCR-ABL^{T315I}). Although second (dasatinib, nilotinib) and third (ponatinib) generation TKIs inhibit imatinib-resistant BCR-ABL mutants, they still fail to overcome disease persistence. Autophagy is a cytoprotective catabolic process activated under stressful conditions and can be inhibited by chloroquine (CQ), a non-specific autophagy/lysosome inhibitor. We have previously shown that TKIs induce autophagy in CML and CQ-mediated autophagy inhibition enhances TKI efficacy. However, fundamental questions still remain unanswered: 1) is autophagy inhibition enhancing TKI efficacy in CML stem/progenitor cells, and what is the effect of TKIs combined with autophagy inhibition on normal stem/progenitor cells. 2) TKIs are known to additionally affect other kinases within the cell, such as PDGFR/c-Kit (imatinib), VEGF/Src (dasatinib), c-Kit/Arg (nilotinib) that could potentially affect autophagy independently of BCR-ABL. **Aims.** We aim to determine synergy between TKIs and specific autophagy ablation and investigate the specificity of TKIs in inducing autophagy by: 1) analysing the effect of TKIs combined with *ATG7* knockdown in CML and normal stem/progenitor cells, 2) investigating if TKIs induce autophagy solely through BCR-ABL inhibition or through other targets as well. **Methods.** In order to evaluate if ablation of autophagy enhances TKI effects, we inhibited autophagy pharmacologically with CQ, and in a specific manner by knocking down *ATG7* (a pivotal autophagy gene) stably, via lentiviral delivery, or transiently, via nucleofection. To achieve this, we developed a pLKO.1-GFP-shATG7 vector-based lentiviral system that reached up to 70% knockdown. A pool of siATG7 clones achieved 50% ATG7 knockdown after nucleoflection.In order to explore if TKI-induced autophagy is exclusively through BCR-ABL inhibition or not, we investigated the activity of the mammalian target of rapamycin (mTOR), a negative autophagy regulator, in BaF3 cell lines (parental, BCR-ABL and BCR-ABL^{T315}) following TKI treatment, by measuring the phosphorylation levels of its downstream effector RPS6. **Results.** Addition of CQ to ponatinib decreased survival of progenitor cells by 12% compared to ponatinib treatment alone (p=0.033) in a clonogenic assay. Additionally, lentiviral inhibition of autophagy enhanced TKI-induced death in CML CD34+ cells. Presence of *ATG7* knockdown in combination with dasatinib resulted in 24% increase in apoptosis and 40% decrease of the colony formation ability compared to TKI treatment alone in K562 cells. Experiments in primary human normal and CML stem cells are under way.Our data suggest that mTOR activity is essentially regulated by BCR-ABL and TKI-induced autophagy in CML cells is mainly driven by BCR-ABL inhibition. However, we observed that dasatinib-induced autophagy could partially be attributed to non-BCR-ABL targets. **Summary and Conclusions.** Autophagy is induced in CML upon TKI inhibition of BCR-ABL and facilitates survival. Our findings suggest that autophagy inhibition enhances TKI-induced cell death in CML stem/progenitor cells and underline the importance of ATG7 as a therapeutic target. Future development of specific autophagy inhibitors in combination with TKIs could target the fraction of persistent CML stem/progenitor cells.

0168

CHARACTERIZATION OF THE BCR-ABL 3'UNTRANSLATED REGION

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Background. The formation of the BCR-ABL gene is observed in a number of leukemias, but is most notably in chronic myeloid leukemia (CML). Recent studies have correlated BCR-ABL "dosage"with disease phenotype. However control of BCR-ABL expression is poorly understood. BCR-ABL transcription is driven by the BCR promoter, and contains the ABL1 3'untranslated region (3'UTR). The 3'UTR is an important regulator of gene expression, and is modulated by a number of factors such as non-coding RNAs and RNA-binding proteins. Therefore, we are investigating the role of the BCR-ABL 3'UTR in its expression. **Aims.** To characterize the BCR-ABL 3'UTR and identify mechanisms that affect BCR-ABL expression. **Methods.** Luciferase assays were used to map regulatory regions within the BCR-ABL 3'UTR. We also performed 3'RACE to look for 3'UTR shortening and measured the half-lives of BCR, BCR-ABL and ABL1. We used bioinformatics to predict microRNA-3'UTR interactions. Luciferase assays and microRNA over-expression in cell lines was carried out to test the bioinformatics predictions. **Results**. Using an ABL 3'UTR luciferase reporter we showed that the 3'UTR was repressive in a number of CML and non-CML cell lines. We also mapped two negative regulatory elements within the 3'UTR. Additionally, we observed post-transcriptional control of the 3'UTR, and that BCR-ABL and ABL have shorter half-lives than BCR. 3'RACE of the ABL1 3'UTR containing genes (ABL1 and BCR-ABL) identified a shortened 3'UTR. In patient samples, this 3'UTR variant was observed in approximately 2% of ABL1 3'UTR transcripts. MicroRNA-29, -30, -196 and 203 were predicted by Targetscan to interact with species-conserved regions of the BCR-ABL 3'UTR. Luciferase assays provide evidence that miR-29, and -196 interact with the 3'UTR. Over-expression of miR-29 and 196b in CML cell lines did not affect BCR-ABL expression. However, miR-196b reduced proliferation of myeloid cell lines. We are also establishing a system to isolate RNA-binding proteins bound the ABL 3'UTR. **Conclusions.** We have shown that the BCR-ABL 3'UTR is repressive and contains two negative regulatory elements. With the repression involving post-transcriptional control, current work is focused on identifying any microRNA or RNA-binding protein interactions with the 3'UTR. We envisage that our research will contribute to a better understanding of the biology of chronic myeloid leukemia and alternative forms of targeted therapy.

0169

BCR-ABL TYROSINE KINASE SUSTAINED MDS1 AND EVI1 COMPLEX LOCUS (MECOM) EXPRESSION IN CHRONIC PHASE CHRONIC MYELOID LEUKAEMIA

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Background. Enhanced expression of MDS1 and EVI1 complex locus (*MECOM*), a proto-oncogene located on chromosome 3q26, is frequently observed in blast crisis (BC) of chronic myeloid leukaemia (CML), implicating this zinc finger transcription factor in disease progression. Shown to have a role in self-renewal, proliferation and the repopulating capacity of murine haemopoietic stem cells in *MECOM* null mice, MECOM's role in CML has not been fully determined. **Aims.** In this study we investigated *MECOM* gene expression, the effect of imatinib mesylate (IM) treatment and the biological activity of this gene in primary chronic phase (CP)-CML CD34+ progenitor cells as well as CML derived cell lines. **Methods.** K562 cells transduced with lentiviral vectors encoding non-target (NT) control or shRNAs targeting *MECOM* were selected by puromycin resistance. Q-PCR for *BCR-ABL*, *MECOM* isoforms and *GAPDH* was performed on an OPTICON 2 DNA engine. Colony forming cell (CFC) assays were performed in Methocult and scored after 12 days in culture. Primary CD34+ cells were enriched by positive magnetically activated cell selection (CliniMACS) to >90% purity and cryopreserved; thawed samples were cultured in IMDM with five growth factors (IL3, IL6, SCF, GCSF and Flt3L). **Results.** Knockdown of *MECOM EVI1* (E) and *MECOM MDS1-EVI1* (ME) isoforms: reduced K562 cell division at low cell density and if cells did divide, they turned over less frequently than parental or NT control cells; inhibited CFC by 34%; and moderately reduced BCR-ABL mRNA and protein expression but not tyrosine kinase activity. IM treatment of K562 in which *MECOM E* and *ME* were knocked down partially restricted differentiation as measured by Glycophorin A+ colonies plucked from CFC. Both *MECOM E* and *ME* transcripts were expressed in CD34+ selected cells of both CP-CML and non-CML (BCR-ABL negative) origin. Indeed CD34+ cells expressed high levels of *MECOM* and both *E* and *ME* transcripts were present in CML cells at similar levels to normal (allogeneic donor) and non-CML cells, with *ME* more abundant than *E*. Further, the splice variants with 324 internal amino acids (*324aa*), Δ*324*aa (missing zinc fingers 6 and 7 and part of the intervening region), *+9aa* (with nine amino acids in the repressor region) and Δ*9aa* were all detectable in CP-CML CD34+ cells at different levels. Furthermore, *MECOM* mRNA and protein expression was repressed by IM treatment of CP-CML CD34+ cells, K562 and KY01 cell lines but had no effect in non-CML CD34+ cells. Summary: The level of *MECOM* gene expression was not elevated by BCR-ABL kinase relative to non-CML and normal primitive haemopoietic cells. However, inhibition of *MECOM* expression by IM establishes for the first time a link between BCR-ABL kinase activity and *MECOM* expression in CP-CML progenitor cells suggesting that BCR-ABL partially mediates its biological activity through MECOM. Our results suggest that BCR-ABL tyrosine kinase activity sustains *MECOM* gene expression in CP-CML progenitor cells contributing to CML pathogenesis.

0171

DASATINIB TREATMENT CAUSES ALTERATIONS IN BONE METABOLISM IN A JUVENILE RODENT MODEL

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Background. In children following treatment failure of chronic myeloid leukemia (CML) by imatinib (IMA) the second generation TKI Dasatinib (DASA; Sprycel®, BMS) represents another option. Similar to IMA this TKI specifically inhibits BCR-ABL but also shows off-target effects on PDGFR and c-FMS involved in bone metabolism (Vandyke K et al. Blood 115:766, 2010; Vandyke K et al. J Bone Miner Res 25:1759, 2010). In adult CML patients disturbances in bone and mineral metabolism (hyperparathyroidism) are reported during IMA therapy while in pediatric patients longitudinal growth retardation was observed (Shima H et al. J Pediatr 159:676, 2011). Pediatric clinical reports on bone side effects exerted by DASA have not been published yet. **Aims.** In a juvenile rat model we investigated the influence of DASA on the metabolism of the growing bone. **Methods.** Juvenile male Wistar rats (milestones of development: end of weaning: age 3 weeks, entering puberty: age 7 weeks) were chronically exposed to varying concentrations of DASA via the drinking water (controls: water; group A: 25 mg/L DASA; group B and C: each 50 mg/L DASA) from age 4 weeks to age 14 weeks. While group A and B were exposed continuously, group C received DASA intermittently (3 days 'ON', 4 day 'OFF'). Animals were sacrificed at age 6 weeks (prepubertal), 8 weeks (pubertal) and 14 weeks (postpubertal), respectively, and the following factors were analyzed: bone length, trabecular and cortical bone mass density (BMD), and bone-specific metabolic serum markers. **Results.** DASA was tolerated at all concentrations and no alterations in animals overall development and behavior were observed. Rats of group A, B, and C exhibited reduced femoral and tibial length and dose-dependently this effect was most pronounced in group B (figure 1). Femoral BMD analysis revealed reduced trabecular BMD postpubertally in all exposed groups while cortical BMD generally was unaffected. Tibial trabecular BMD was slightly decreased at prepubertal age in groups A and B but normalized under ongoing treatment. Tibial cortical BMD was not altered. Also no changes were found in vertebrae. Biochemical markers of bone resorption (CTX-I and TRAP) indicated no significant differences. Bone formation marker osteocalcin was reduced prepubertally by 20 % (group A), 30 % (group B), and 28 % (group C), respectively, followed by normalization during puberty. Parathormone and inhibin B levels remained normal. Growth hormone levels were found elevated during DASA exposure, however, IGF-BP3 was highly significantly lowered in all groups and at all ages. **Conclusions.** This juvenile rodent model is useful to mimic side effects of TKI treatment observed in still growing pediatric patients. As has been shown before for IMA (Tauer JT et al. EHA 2011 Abstract #0670; Tauer JT et al. ASH 2011, Abstract #3741) DASA also alters bone modeling and mandates pediatricians to carefully monitor osseous side effects in not yet outgrown patients. Suttorp M et al. Hematology ASH Educ Program 2010:368; Suttorp M et al. Curr Hematol Malig Rep 2012, in press.

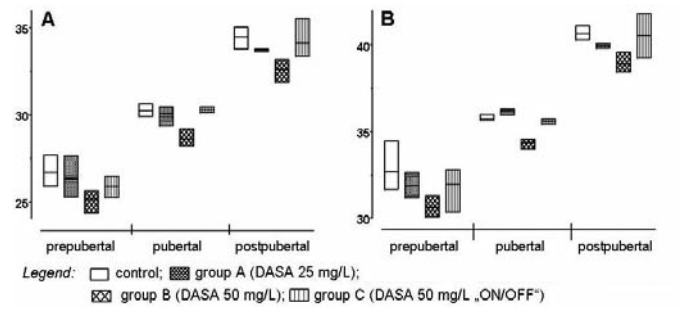

Figure 1. Length (mm) of the A) femur and B) tibia.

0172

AUTOPHAGY GENES SUPPRESSED IN CML ARE UP-REGULATED FOLLOWING BCR-ABL INHIBITION WITH TKI

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Background. Treatment of Chronic Myeloid Leukaemia (CML) has been radically transformed by introduction of Tyrosine Kinase Inhibitors (TKI), imatinib, dasatinib and nilotinib, which are capable of inhibiting constitutively active BCR-ABL kinase. But survival of residual CML stem cells after TKI treatment is a major obstacle for the elimination of the disease in patients. We have shown that autophagy is one of the mechanisms for survival of CML stem cells following TKI treatment. In vitro, autophagy can be inhibited by the lysosomotrophic agent chloroquine that inhibits lysosomal degradation of autophagosomes [1], [2]. CHOICES, (CHlorOquine and Imatinib Combination to Eliminate Stem cells), a randomised Phase II trial is ongoing to evaluate the response to autophagy inhibition in patients (ClinicalTrials.gov Identifier: NCT01227135). Autophagy can be induced as a survival mechanism following many conventional chemo and radio therapies. Therefore understanding of transcriptional regulate: on of this process is critical for targeted therapy against autophagy.AimAutophagy serves as a rescue mechanism in CML cells following TKI treatment. The aim of this project was to identify autophagy genes that were differentially regulated in CML versus normal cells at baseline and in response to TKI.MethodIn this study we have investigated the expression of a panel of 14 autophagy genes using Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) in primary CML and non-CML CD34+ cells and in CML cell lines. The protein expression was analysed by western blot.ResultsWe demonstrated that BCR-ABL+ CML progenitor cells have significantly lower expression of autophagy genes compared to non-CML progenitor cells. Following TKI mediated inhibition of BCR-ABL kinase activity, expression of key autophagy genes such as Ulk1, Ulk2, Vps34 and GabarapL was significantly up-regulated. Constitutive expression of BCR-ABL was sufficient to maintain the low basal expression of these genes in CML cell lines even after growth factor withdrawal for 24 hours, but growth factor withdrawal combined with TKI treatment induced the expression of the same genes in these cells. In a T315I mutant cell line where BCR-ABL is not inhibited by conventional TKIs the genes were only up-regulated following treatment with third generation TKI, Ponatinib, known to effectively inhibit T315I mutant BCR-ABL kinase activity.ConclusionTaken together it appears that BCR-ABL suppresses the transcription of autophagy genes in CML cells. Autophagy is up-regulated following TKI mediated inhibition of BCR-ABL, not only as indicated by protein markers, but also results in significant induction of transcription of autophagy genes. The study will allow us to identify critical genes controlling TKI mediated autophagy that can be targeted for future pharmacotherapy in CML and other malignancies for wider application.

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0173

NUP98/HOXA13 FUSION GENE AND HIGH MSI2 GENE EXPRESSION IN BLASTIC PHASE CHRONIC MYELOID LEUKEMIA

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Background. In hematopoietic malignancies, such as acute myeloid leukemia (AML), myelodysplastic syndromes, chronic myeloid leukemia in blast crisis (BC-CML), T-cell acute lymphoblastic leukemia, recurrent balanced translocations and inversions cause *NUP98* (11p15) to fuse with a partner gene. All fusion transcripts maintain the 5'-end of *NUP98* and the 3'-end of the partner gene, at least 28 of which have been identified to date. They are either homeobox (HOX), with a homeodomain region, or non-homeobox genes. NUP98/

HOX proteins retain the homeodomain with its DNA binding ability. Some also retain a short ANWL motif, which binds the transcriptional cofactors of the PBX family. The best characterized *HOX* partner is found in the *NUP98-HOXA9* fusion from a *t*(7;*11*)(p15;p15) and was first identified in AML (Nakamura et al Nature Genetics 1996). Subsequently, cooperation of NUP98/HOXA9 with BCR-ABL (Moore et al. *Ann.N.Y.Acad.Sci.* 2007) was proven. Recently Ito et al. (*Nature* 2010)and Kharas et al. (*Nature Medicine* 2010) showed that murine *Nup98/HoxA9* binds the *Musashi2* gene transcription start site, leading to over-expression, downstream NUMB-NOTCH1 pathway deregulation, loss of myeloid cell differentiation and increased proliferation. **Aims.** To investigate *MSI2* gene expression regulation in BC-CML bearing *NUP98/ HOXA13* fusion. **Methods and Patients.** Conventional Cytogenetics and FISH studies were performed on peripheral blood cells at diagnosis of BC-CML in a 39-year old man. Karyotype was 46,XY,t(7;11) (p15;p15),t(9;22) (q34;q11). FISH and RT-PCR confirmed the *BCR/ABL1* fusion. Given the 11p15 breakpoint, we performed a break-apart FISH assay with clones RP11-348A20 and CTD-3234F16 for *NUP98* gene (Gorello et al *Haematologica* 2008). The 7p15 breakpoint was investigated with probes RP1-170O19 and RP1-167F23 spanning the *HOXA* cluster genes. Nested-PCRs used *NUP98*-specific forward primers and specific reverse primers for the hypothetical partner genes: *HOXA9, HOXA11* and *HOXA13*.qRT-PCR was performed on the patient's cryopreserved bone marrow RNA sample. Additional samples included 13 BC-CML, 3 CP-CML, 4 AML (3 NPM+FLT3+ and 1 NPM+FLT3-) Controls were 3 non-neoplastic bone marrow samples. Quantitative PCR was performed using TaqMan probes (Applied Biosystems) Hs00292670_m1, for *MSI2*, and Hs00245445_m1 for the endogenous reference control, *ABL1*. Expression levels were normalized with the Universal Human Reference RNA (Stratagene). **Results.** Three splicing variants of the in frame fusion transcript *NUP98/HOXA13* were identified, all with the breakpoint between exon 16 (nt 2322) of *NUP98* and exon 2 (nt 952) of *HOXA13* gene. *MSI2* was over-expressed in the *NUP98/HOXA13* patient (6.21) another BC-CML case with complex karyotype (5.27), and 2/4 AML cases, both NPM+FLT3+ (6.69 and 6.92). Median values were: 1.96 for BC-CML, 3.14 for CP-CML and 3.55 for controls. **Conclusions.** We show for the first time that, in addition to *NUP98/HOXA9*, also the *NUP98/HOXA13* fusion gene underlies *Musashi2* over-expression in BC-CML. As *MSI2* was over-expressed in another BC-CML case with a complex karyotype and no evidence of t(7;11), we are now investigating its molecular lesions. *MSI2* over-expression in two NPM+FLT3+ AML confirmed previous results in a subset of AML (Griner et al. *Cancer Biology and Therapy* 2010). High *MSI2* expression could be one critical pathway in acute myeloid proliferation and may underlie BC progression in Ph-positive CML.

0174

CD34+ HIGH RESOLUTION KARYOTYPING OF SOMATIC LESIONS AT CHRONIC PHASE CML DIAGNOSIS AND ASSOCIATION WITH SOKAL RISK SCORE: A PRELIMINARY PILOT STUDY. ON THE BEHALF OF THE FI-LMC GROUP

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Background. Although most patients in chronic phase CML (CP-CML) respond to tyrosine kinase inhibitor therapy, the kinetic of response and - for patients reaching stable complete molecular response - the probability to maintain response after therapy cessation, are different. To date, clinical heterogeneity of CML is only assessed by pre-treatment scoring such as the Sokal score. It is believed that this clinical heterogeneity depends on the inherent nature of the disease and may be related to genetic heterogeneity of CML progenitors at diagnosis. **Aims.** To clarify the biological significance of Sokal score and to increase predictive value of pre-treatment scores, especially for patients of intermediate risk, using high-resolution genetic characterization. **Methods.**We collected 100 CD34+/fibroblasts paired samples at CP-CML diagnosis from bone marrow aspirates, and have performed Genome-Wide Human SNP 6.0 array (Affymetrix) for SNP-A karyotyping analysis on a subset of 40 pairs. **Results.** For this pilot study 40 of 100 patients have been analyzed (14 low risk, 22 intermediate risk, and four high risk patients). We have detected somatic alterations widely distributed among all chromosomes: 206 losses; 225 gains; 396 loss of heterozygoty/ uniparental disomy [LOH/UPD]. These lesions are of relatively small size, ranging from 100 to 1000 Kb, and from 1041 to 9000 Kb for gains/loss and LOH/UPD, respectively.Statistical analysis revealed lack of significant correlation between Sokal score and age, and type of alteration (i.e., LOH, gains, and losses). Interestingly, a lower Sokal score tends to have more genetic lesions, and vice versa (p=0.068). Of note, the number of patients with high Sokal score is very low in this preliminary analysis, and thus not allowing a definitive conclusion. However, this observation suggests that it is not the number of lesions (probably randomly distributed) but likely the type of affected region which may be relevant. Indeed, we have found 9q22.32-22.33 and 17p11.2 recurrent UPD among two out of the four high scored patients, both regions include candidate genes known to be involved in tumorogenesis or cell proliferation. Interestingly, the 17p11.2 region is also subject to parental imprinting. **Conclusions.** Our preliminary results confirm the genetic heterogeneity of CML at diagnosis with genetic instability-related alterations randomly distributed. But the inherent nature of CML (illustrated by the Sokal score) may more likely be related to the type of alteration, with only few relevant alterations related with higher pre-therapeutic score, rather than to the number of genetic lesions (i.e. level of genetic instability). Next generation sequencing strategies of candidate genes within these recurrent UPD regions, if these results are confirmed on the entire cohort with increased number of high Sokal score, will then be further investigated.

0175

THE MATRICELLULAR PROTEIN CCN3 REDUCES NOTCH SIGNALING IN CHRONIC MYELOID LEUKEMIA

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Background. Notch is a key regulator of hematopoiesis. Active Notch maintains hematopoietic stem cells in an undifferentiated state and inhibition of Notch signaling is required for differentiation to proceed. Deregulated Notch has been associated with Leukemia, although its role in Chronic Myeloid Leukemia (CML) is not well established. Previously, we reported BCR-ABL downregulation of the matricellular protein Ccn3 in CML; Ccn3 is a Notch ligand. This study examines the Notch-Ccn3 signaling axis in CML. **Aims.** To characterize the Notch-Ccn3 signaling pathway in CML. **Methods.** Western blotting was used to determine Notch1 and Ccn3 expression in CD34+ progenitors, mononuclear cells and neutrophils from CML and normal bone marrow (n=5). K562, KCL22 and LAMA cells (none of which express significant Ccn3 as a consequence of Bcr-Abl activity) were used as CML model systems. K562 cells stably overexpressing CCN3 (K562/CCN3) were used to study signaling mechanisms. Using siRNA, BCR-ABL was silenced in K562 cells and expression of Notch1 full length (FL), Notch1 intracellular domain (ICD), downstream targets c-Myc and Hes1 were evaluated. CML cell lines K562, K562/CCN3, KCL22 and LAMA were treated with gamma secretase inhibitor (GSI) to block Notch signaling (25 nM-1 μM) and viability measured by CellTiter-Glo®. Additional functional readouts were clonogenic assays and cell cycle analysis. All experiments were performed in triplicate. **Results.** Normal bone marrow cells homogeneously express Notch1, whilst Ccn3 is moderately expressed in CD34+ and mononuclear cells and reduced in neutrophils, consistent with a role for Ccn3 inducing myelopoietic differentiation. In CML, Notch1 is highly expressed in CD34+, mononuclear cells and neutrophils whereas Ccn3 is absent in CD34+ cells and neutrophils. Silencing BCR-ABL in K562 cells reduced Notch-FL and completely inhibited Notch-ICD cleavage, c-Myc and Hes1 ($p<0.001$). Notch1 mRNA levels were comparable in K562 and K562/CCN3 cells whereas Notch FL, Notch-ICD and Hes1 were reduced in K562/CCN3 cells ($p<0.01$). Co-immunoprecipitation showed Ccn3 interacting with Notch extracellular domain. GSI treatment of K562, KCL22 and LAMA cell lines had no effect on cell viability but reduced K562/CCN3 proliferation (100 nM GSI, 50.9±6.75 %, $p<0.01$). GSI treatment significantly decreased expression of Notch-FL and Notch-ICD in K562/CCN3 cells but in K562 cells Notch-FL was stabilized and Notch-ICD unchanged. Cell cycle analysis showed a subtle but significant increase in the

sub G0 population in K562/CCN3 cells (~2 fold, p<0.05) and notably formed fewer colonies with all concentrations of GSI (25 nM GSI, 11.6±0.43 in K562/CCN3 and 80.6±1.38 in K562 cells, p<0.001). Treatment with recombinant Ccn3 (rCcn3) reduced Notch-ICD and Hes1 expression in K562 cells (p<0.01). rCcn3 sensitized K562 cells to GSI with 30.2±1.04% reduction in viability (100 nM GSI, p<0.01). **Conclusions.** In CML, Bcr-Abl upregulates Notch1 simultaneously downregulating Ccn3, which disrupts the Notch-Ccn3 axis. Our study demonstrates Ccn3 as a negative regulator of Notch in CML and suggests Notch-Ccn3 signaling as an essential pathway maintaining normal hematopoietic function. Further understanding of Notch-Ccn3 axis may provide basis for novel treatment strategies in CML.

0176

UP REGULATION OF PEROXIREDOXIN 2 IS CORRELATED WITH DISAPPEARANCE OF PHILADELPHIA CHROMOSOME IN THE BONE MARROW OF CML PATIENTS DURING IMATINIB THERAPY

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Background. Several lines of evidence associate ROS and antioxidant enzymes, which appear to play a major role not only in leukemogensis but display therapeutic implications in CML: bcr-abl tyrosine kinase induces the production of ROS in hematopoietic cells (Sattler et al 2000). Previously, our Lab. had found that peroxiredoxins (Prxs) are a ubiquitous family of multifunctional antioxidant thioredoxin-dependent peroxidases that have roles in the reversible inactivation of PTPs and PTEN in cells stimulated by growth factors and protect cells against oxidative stress and modulate intracellular signaling cascades (Rhee et al. Current Opinion in Cell Biology 17;183-189,2005). Recently, Agrawal-Singh et al (Blood 2011, Dec) had reported that PRX2 may be a tumor suppressor gene based on genomic wide analysis in myeloid leukemia primary cells. **Aims.** In this study, we investigated the changes of the levels of H_2O_2-removing enzymes (ie Prxs, glutathione peroxidase 1 (Gpx1), and catalase) during IM therapy in newly diagnosed CML and in the Ph positive K562 cell line with Imatinib treatment *in vitro* as well. **Patients and Methods.** Newly diagnosed CML BM samples were analyzed. This study was approved by the institutional review board. Patients were given written informed consents according to institutional guidelines. 400 mg or 600 mg of Imatinib was given to the chronic phase and accelerated/blast crisis samples respectively. Pairs of bone marrow sample at the diagnosis and at cytologic complete remission status of CML patients were evaluated for detection of intracellular ROS using dichlorodihydrofluorescein diacetate (DCFH-DA), the Immunohistochemical expression of PRX on single cell level and immunoblot assay for the expression of Prx1-6 and Gpx1. K562 cells were treated with Imatinib for 48 hours and RT-PCR and Immunoblot assay for PRX1-2 were performed. **Results.** Samples of newly diagnosed CML patients showed significantly decreased levels of Prx 2, but revealed increased level of catalase. As the level of Philadelphia chromosomes decreased with IM treatment, the expression levels of Prx II and catalase were restored to the levels of normal individuals. Interestingly, the Immunohistochemical expression of PRX on single cell level was well correlated with western blot results of PRX. Meanwhile, with the treatment of Imatinib into K562 cell line for 48hrs at 10 , there were significant elevations of PRX2, which may be supportive findings for the observations on CML patients during Imatinib treatment *in vivo*. In contrast, there were no obvious changes of PRX1 or PRX3 on protein level in K562 cells after treatment of Imatinib.**Conclusions.** Decreased Prx 2 and elevated catalase levels at the time of diagnosis are closely correlated with the elevated bcr/abl kinase level in newly diagnosed CML. The aberrant expression of those antioxidant enzymes were back to the level of normal individuals after IM treatment. Understanding the molecular mechanisms of changes on antioxidant may be potent tools to develop more effective new drugs in CML patients especially for Imatinib resistant patients.

0177

INVOLVEMENT OF HEME OXYGENASE 1 IN THE MECHANISMS OF RESISTANCE TO IMATINIB MESYLATE IN CHRONIC MYELOID LEUKEMIA CELLS

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Chronic myeloid leukemia (CML) is a stem cell disease in which BCR/ABL promotes the survival of leukemic cells. Identification of imatinib mesylate (IM) as a potent inhibitor of the Abl kinase and the subsequent findings that this compound displays growth inhibitory and pro-apoptotic effects in Bcr-Abl+ cells has deeply conditioned CML treatment. Unfortunately the initial striking efficacy of this drug has been overshadowed by the development of clinical resistance. Although the majority of Ph+ CML patients benefit from IM treatment, a substantial number of patients are either initially refractory to treatment or develop resistance during the course of treatment. A wide variety of molecular mechanisms can underlie resistance mechanisms. In the recent years, heme oxygenase-1 (HO-1) expression has been reported as an important protective endogenous mechanism against physical, chemical and biological stress and this cytoprotective role has already been demonstrated for several solid tumors and acute leukemia. To investigate the effect of HO-1 expression on cell proliferation and apoptosis in chronic myeloid leukemia cells, K562 and LAMA-84 cell lines were incubated for 24h with IM (1μM) or in combination with an inducer or inhibitor of HO-1 (Hemin and SnMP). In addition, cells were also treated with HO byproducts, bilirubin and carbon monoxide (CO), or with a protease inhibitor (Ed64). After pharmacological treatments we evaluated cell viability and HO-1 expression by real-time PCR. Pharmacological induction of HO-1 was able to overcome the effect of imatinib. The cytoprotective effect of HO-1 was further confirmed after silencing HO-1 by siRNA. Interestingly, neither bilirubin nor CO were able to protect cells from IM-induced toxicity. The protective effect of HO-1 was mitigated by the addition of Ed64, preventing HO-1 nuclear translocation. We also analyzed 96 kinase genes using *TaqMan® Low Density Array Human Kinases Panel* and found that induction of HO-1 in combination with IM increased seven different kinases. Finally, IM was able to increase the formation of cellular reactive oxygen species. This effect was reversed by HO-1 induction or the addition of N-acetylcisteine. In conclusion, the protective effect of HO-1 on IM-induced cytotoxicity does not involve its enzymatic by-products, but rather the nuclear translocation of HO-1 following proteolytic cleavage. Migration of HO-1 into the nucleus activates several kinases which may be responsible for mitogenic signals activation.

0178

DIFFERENTIAL EXPRESSION OF BACULOVIRAL IAP REPEAT-CONTAINING FAMILY GENES IN CHRONIC MYELOID LEUKEMIA AS POTENTIAL MARKERS OF DISEASE PROGRESSION

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Background. Introduction of tyrosine kinase inhibitors (TKI) into clinical practice transformed chronic myeloid leukemia (CML) into truly chronic disorder. Although in the majority of cases remissions are durable, there is still a significant group of patients who develop resistance to the treatment and progress to blast crisis. Hence, there are still some unsolved clinical problems, including lack of reliable factors predicting rise in BCR/ABL level and disease progression. B*aculoviral* IAP repeat-containing (BIRC) is a family of eight functionally- and structurally-related proteins, which serve as endogenous inhibitors of apoptosis. Overexpression of various *BIRC* genes has been associated with cancer progression, multidrug resistance, poor prognosis and short survival in several types of neoplasms including hematological malignancies. In CML blast crisis survivin (*BIRC5*) and XIAP (*BIRC4*) were upregulated in comparison to chronic phase. However, there is no data on the role of other *BIRC* family members in prediction of CML progression. **Aims**. Analysis of the expression of *BIRC* genes at various stages of CML as potential markers allowing predicting disease progression in patients treated with TKI.MethodsRelative expression of family of *BIRC* genes was tested using RT-qPCR in sequential samples of cDNA from peripheral blood leukocytes (PBL) obtained from CML patients at various stages of the disease (from diagnosis to progression to blast crisis) simultaneously with regular *BCR/ABL* expression monitoring. Blood samples were taken after informed consent. The results were confirmed in larger group of cDNA samples obtained from patients in chronic phase or blast crisis as well as healthy blood donors. RT-qPCR experiments were performed according to MIQE guidelines. The changes of BIRC proteins level were observed in West-

ern blotting experiments performed on PBL lysates from CML patients and healthy blood donors.ResultsAs previously described, *BIRC5* expression was upregulated in patients in blast crisis in comparison to patients in chronic phase. In turn, the other *BIRC* genes were differentially expressed and did not follow expected pattern (Figure 1), i.e. *BIRC1* expression was significantly increased in blast crisis, the expression of *BIRC2*, *BIRC3*, *BIRC4* and *BIRC6* was barely changed in the course of the disease, whereas *BIRC7* expression was at the limit of detection. Unexpectedly, massive drop of *BIRC8* expression was observed in blast crisis samples in comparison to samples obtained from patients in chronic phase and healthy blood donors. Downregulation of *BIRC8* in blast crisis was observed also at the protein level. **Summary.** This is the first study covering a complete set of *BIRC* gene family expression in various stages of CML. Among the studied genes, expression changes in blast crisis in comparison to chronic phase were observed for three genes: a significant upregulation of *BIRC1* and *BIRC5* as well as downregulation of *BIRC8*. Hence, after validation, they may serve as markers of progression in CML. However, elucidation of the role of *BIRC8* downregulation in the progression of CML requires further studies.

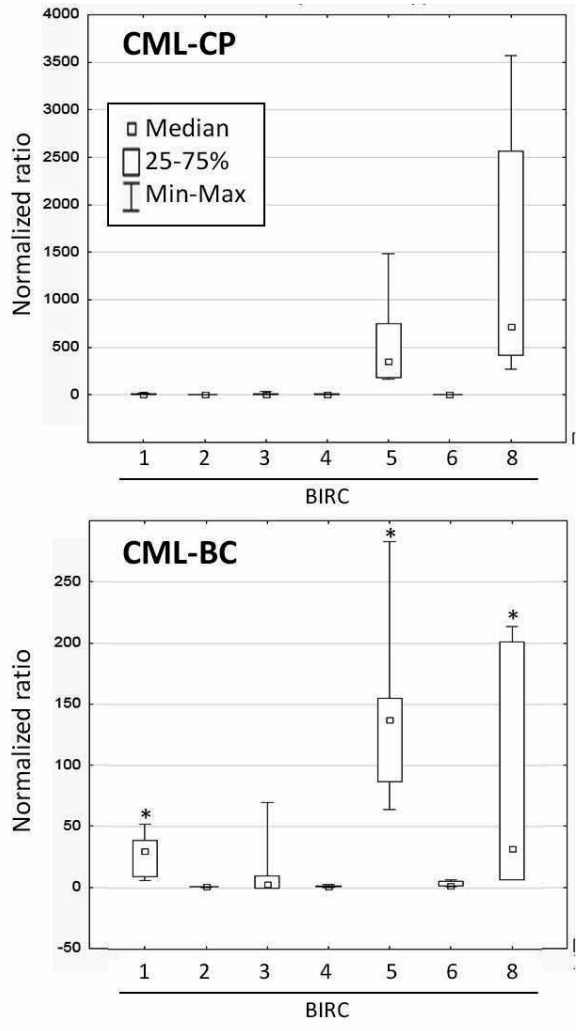

Figure 1. Expression of BIRC genes in chronic phase (CML-CP) and blast crisis (CML-BC). *p<0.05 vs. expression in CML-CP

0179

COMPLEX TRANSLOCATIONS IN CML: CHROMOSOMES INVOLVED AND MECHANISMS OF DEVELOPMENT

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Background. Philadelphia chromosome is a main marker of the chronic myelogenous leukemia (CML) and occurs as a result of reciprocal translocation t(9;22)(q34;q11). In about 10-15% of the cases formation of Ph-chromosome is caused by complex translocations involving one or more additional chromosomes. *Aim* of this study was to determine frequency of complex translocations in CML and chromosomes involved. **Methods.** Cytogenetic investigations were performed in 212 patients with CML. Methods used were conventional cytogenetics and fluorescent *in situ* hybridization (FISH). *Results.* Of 212 patients with cytogenetically confirmed diagnosis of CML, 192 (91%) had Ph-chromosome formed by the standard translocation t(9;22)(q34;q11), whereas other 20 (9%) - had complex translocations. In 18 patients of the latter group complex translocations were revealed in all Ph-positive metaphases; other 2 patients had cells with both typical and complex translocations. In all of the 20 cases of complex translocations chromosomes 9 and 22 were involved with breakpoints typical for CML - 9q34 and 22q11. Except for the typical chromosomes 9 and 22, in 17 of complex translocations only 1 additional chromosome was involved; in 1 case - 3 chromosomes; in 1 more case - 4 chromosomes were engaged. The origin of additional chromosome was not determined in 1 case. Chromosome 2 was found to participate in three cases of complex translocations; chromosomes 1, 3, 4 and 17 - in 2 cases; and chromosomes 5, 6, 8, 9, 10, 11, 12, 13, 14, 16 and 19 - were involved in 1 case. The following breakpoints were engaged in the detected complex translocations: 1p36, 1q32, 2p13, 2q13, 2q36, 3p21, 3q25, 4p16, 4q12, 5q31, 6p21, 8q24, 9q22, 10q24, 11q13, 12q22, 13q13, 14q24, 16q23, 17p11, 17q23, 19q13. In one of the cases exchange of the material between der(22) and chromosome 5 was revealed which resulted in atypical morphology of der(22) and localization of *BCR/ABL* gene on derivative of chromosome 5. In this case gene *ABL/BCR* was located on der(9). It may be assumed that in 3 cases the mechanism of formation of complex translocations (including cells with different types of translocations as well as the case of t(9;22;5)(q34;q11;q31) resulting in atypical localization of *BCR/ABL*) was a two-step process. Whereas in the remaining 17 cases it was a one-step event. **Conclusions.** In our study frequency of complex translocations was found to be 9%, which is comparable to the data reported in the literature. It was found that complex translocations apart from chromosomes 9 and 22 can involve almost all chromosomes of karyotype with various breakpoints engaged. However, most frequent additional chromosomes were chromosome 2 (15% cases), 1, 3, 4 and 17 (10% of complex translocations each). Complex translocations involved more than 1 additional chromosome in 10% of cases. Probability of both one-step and two-step mechanisms of complex translocation occurrence was shown with respective frequency of 85% and 15%. Presence of secondary complex translocation may result in modifications of morphology of der(22) and localization of *BCR/ABL* gene and may be the sign of clonal evolution.

0180

COMPARATIVE PHENOTYPIC ANALYSIS OF NK RECEPTORS ON 25 CP-CML PATIENTS WITH SUSTAINED COMPLETE MOLECULAR RESPONSE (CMR) AND MAJOR MOLECULAR RESPONSE (MMR)

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Background. The critical role of BCR-ABL kinase activity in chronic myeloid leukemia (CML) pathogenesis has prompted tyrosine kinase inhibitors (TKIs) to become the gold standard of CML treatment. Although targeted therapy effectively induces and sustains cytogenetic and molecular responses, low levels of disease often persists, and TKIs can be safely discontinued only in a small subset of patients. Why a proportion of CMR patients do no relapse remains poorly understood and immunologic control of leukemic clone has been advocated. Several studies demonstrated that NK cells exert antileukemic activity through “missing self”recognition, which is regulated by a balance between NK inhibitory or activatory receptors signals. Previous reports have outlined the role of NKG2D in susceptibility of CML to NK cell-mediated cytotoxicity, which is par-

adoxically counterbalanced by imatinib negative modulation. **Aims.** The aims of the study were to assess whether a specific pattern of NK receptors (NKR) correlated with the achievement of complete or major molecular response. In addition, we investigated the presence of distinct NKR profiles within CMR patients group. **Methods.** Peripheral blood samples were collected from 25 CP-CML patients treated with imatinib. 10 patients with sustained CMR (median age 52 years, 35-75; mean disease duration 84 months; mean treatment duration 68 months, 19-117; mean response duration 52.7 months, 24-87), 15 patients with MMR (median age 56 years, 33-73; mean disease duration 68 months; mean treatment duration 57 months, 17-96; mean response duration 23.6 months, 12-42).NK receptors expression (Killer Immunoglobulin-like Receptors, KIR: p70, p140, p58/p50; Killer Lectin-like Receptors, KLR: CD94, NKG2A, NKG2C/A, NKG2D; Natural Cytotoxicity Receptors, NCR: NKp30, NKp44, NKp46, NKp80; Co-receptors: 2B4; LIR1/ILT2, GPR56) was analyzed by flow cytometry in CD3-/CD16+ subset.4. **Results.** WBC count, lymphocyte count and NK cell percentage were comparable between the two groups. No statistical differences in KIR profile were observed; interestingly, lower expression of p70 was documented compared to healthy controls (% positive cells: 3.9 and 5.4 respectively vs 17.1); NCR receptors were uniformly expressed in the two groups and were similar to healthy controls; lower expression of KLR NKG2A was observed when compared with normal population.As we observed a wide intragroup variability of NKG2D (33% of CMR patients were NKG2D negative) we performed a subanalysis of CMR patients based on NKG2D expression. Remarkably, activating receptors were preferentially expressed in NKG2D+ group: NKG2C (12.5% vs 7%; p=0.014), 2B4 (50% vs 38%; p=0.019), NKp46 (9.5% vs 4.5%; p=0.021). **Conclusions.** Although no NK receptor profiles seems to correlate with achievement of CMR or MMR (probably because these are not really distinct populations since some MMR patients will obtain CMR with time), we identified two subpopulations of CMR patients, based on NKG2D expression, with distinct activating receptor profile. This data suggest that NKG2D mediated cytotoxicity and, possibly, resistance to imatinib negative modulation, might account for CML residual disease control by NK cells in a subset of CMR patients. We are planning to evaluate the prognostic value of NKG2D expression in terms of complete molecular response maintenance after therapy discontinuation.

0181

EXPRESSION OF GLICOPROTEIN PGP-170 BY THE HEMOPOIETIC PERIPHERAL BLOOD AND BONE MARROW CELLS IN THE CML PATIENTS WITH DIFFERENT RESPONSE TO TYROSINE KINASE INHIBITORS THERAPY

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Background. Despite the successes of tyrosine kinase inhibitor (TKI) therapy, some patients with newly diagnosed chronic phase (CP) of chronic myeloid leukemia (CML) have inadequate response to treatment. The main causes of resistance are BCR-ABL mutations and clonal evolution. However, there are other mechanisms of resistance including hyperexpression of transmembrane glycoprotein Pgp-170, that is a a marker of multidrug resistance. **Aims.** the aim of study was to determine the features of expression of glycoprotein Pgp-170 in CML patients treated by TKI. **Methods.** We included 46 patients with CP-CML in study. Median age of patients was 35.3 years (range 19-65). Diagnostics and monitoring of CML were perfomed on the basis of cytogenetic examination of bone marrow cells by G-banding, and also molecular-genetic research of bone marrow and peripheral blood. The efficiency of TKI application was evaluated after 12 months of therapy. 36 patients have received imatinib (40% of them as the first line therapy), 10 patients have taken nilotinib (20% of them as first line therapy, the others have taken nilotinib after failure of imatinib treatment). The patients with suboptimal response to therapy and treatment failures were classified as resistant patients. The immunological studies were conducted on a FACscan flow laser cytometer. The number of $CD33^+$, $CD34^+$ cells in peripheral blood and bone marrow expressing Pgp-170 in CML patients was determined. **Results.** Results of study suggested that the number of $CD33^+$ hemopoietic cells with co-expression of transmembrane glycoprotein Pgp-170 was increased in CML patients who had resistance to TKI comparing with patients with optimal response on therapy ($p<0.05$). Comparing analysis has shown that in CML patients with resistance to therapy the number of $CD34^+Pgp\text{-}170^+$ hemopoietic cells in bone marrow and peripheral blood was also increased ($p<0.05$). The direct correlation between the number of $CD33^+$ $Pgp\text{-}170^+$ and Ph^+ bone marrow cells have been determined ($r = 0,893$, $p<0,0001$). **Conclusions.** The results of our study confirm the role of Pgp-170 in the development of resistance to TKI therapy in CML patients treated by TKI.

0182

IS DETECTION OF CYP3A5*3 ALLELE IN CHRONIC MYELOID LEUKEMIA PATIENTS PRIOR TO TREATMENT WITH IMATINIB MESYLATE RELEVANT?

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Background. Imatinib mesylate (IM) is known to be metabolized by CYP3A subfamily members of cytochrome P450 family. When expressed, CYP3A5 can represent up to 50% of CYP3A protein and its inactivation can lead to significant variation of CYP3A mediated IM metabolism. Inactive allele CYP3A5*3 is predominant in Caucasian population. This single nucleotide polymorphism (SNP) A6986G (rs776746) forms cryptic splicing site and leads to incorporation of intron into mRNA, premature termination of translation and expression of truncated protein. Active allele CYP3A5*1 is very rare in Caucasian population, but there are significant differences in distribution of CYP3A5 allelic variants in different ethnic groups. **Aims.** To determine frequency of CYP3A5*3 allele in our cohort of IM-treated patients and assess the relevance of CYP3A5*3 allele detection prior to IM treatment. **Methods.** We retrospectively analyzed therapeutical response to IM (400 mg /day) according to European LeukemiaNet (ELN) recommendations in a cohort of 64 patients diagnosed in chronic phase of CML. Patients with treatment failure due to mutations in kinase domain of ABL were not included. CYP3A5*3 was detected by TaqMan SNP genotyping assay. IM plasma levels were detected by liquid chromatography from samples taken 24 hours (+/- 2 hours) after the last imatinib dose. **Results.** Our cohort consisted of 58 CYP3A5*3 homozygotes, 5 heterozygotes and 1 wild type homozygote, leading to the frequency of allele different than CYP3A5*3 5.7%. Major molecular response at 18 months after diagnosis was not associated with CYP3A5*3/*3 genotype ($n = 63$, $p = 0.191$) but heterozygous or wild type individuals for CYP3A5*3 allele showed a trend towards failure of complete cytogenetical response at 12 months ($n = 64$, $p = 0.053$). Plasma levels of IM were not altered by CYP3A5*3/*3 genotype ($n = 56$, $p = 0.656$). **Conclusions.** Our study did not confirm any association of heterozygous or wild type genotype for CYP3A5*3 allele and suboptimal response or failure of IM treatment according to ELN but heterozygous or wild type individuals for CYP3A5*3 allele displayed a trend towards failure of complete cytogenetical response at 12 months after diagnosis. Thus, cheap and fast genotyping of CYP3A5*3 in Caucasian population could be relevant but further study with larger patient cohorts are needed to confirm our findings. This study was supported by research project MZO 00179906 from Ministry of Health, Czech Republic.

Chronic myeloid leukemia - Clinical 1

0183

T315I MUTATIONS ARE CLUSTERED WITH ADVANCED PHASES CONTRARY TO OTHER ABERRATIONS IN CML PATIENTS RESISTANT TO TKI THERAPY

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Background. Various mechanisms of resistance to tyrosine kinase inhibitors (TKIs) in chronic myelogenous leukemia (CML) patients were described. Among them, mutations within *BCR-ABL1* kinase domain are the most frequently studied, since they affect binding of TKI molecule. However, mechanisms leading to subsequent progression are still not fully elucidated. Inhibition of BCR-ABL1 by imatinib was suggested to induce p53 pathway, therefore, p53 inactivation could possibly influence therapy response. Beside this, mutations in genes involved in myeloid transformation (e.g. *ASXL11* and *CBL*) were identified, and their role in disease progression is under investigation. **Aims.** We analyzed genomic aberrations and mutations in *BCR-ABL1*, *TP53*, *ASXL1* and *CBL* genes in patients with primary or acquired resistance to TKIs therapy. **Methods.** Only patients that lost or never achieved major cytogenetic response were included in the study. Mutations in *BCR-ABL1* kinase domain were detected using direct sequencing and T315I-specific ligation PCR. *TP53* mutational status of exons 4-10 was examined by functional analysis (FASAY) coupled to direct sequencing. Mutational analysis of *ASXL1* (exon 12) and *CBL* (exons 8, 9) genes was performed by direct sequencing of gDNA. Genomic changes were detected using conventional metaphase cytogenetics and Affymetrix 2.7M Cytogenetic arrays. **Results.** In total, 26 TKI-resistant CML patients were investigated. 6 patients were examined at the time of blast crisis and 4 in the accelerated phase. In 12 patients *BCR-ABL1* mutation was identified at the time of genomic analyses, among them, 6 patients carried mutation T315I. Remaining patients (n=20) were additionally examined using T315I-specific ligation PCR, and this mutation was identified in 2 patients thereafter. Remarkably, from patients with T315I mutations (n=8), 7 were in advanced disease phase. Additional genomic aberrations were identified in 13/26 of patients. The detected changes included commonly observed large alterations (+8, -Y, i(17q)), cryptic copy number changes and uniparental disomy. Disruption of the p53 pathway was observed in 3 patients: one patient had *TP53* gene deletion caused by i(17q), one harbored biallelic deletion of *CDKN2A* that codes for a protein stabilizing p53 protein, and in one patient a *TP53* mutation was detected. The *TP53* mutation was present after progression to the blast crisis and was not detected in chronic phase, when TKIs resistance was manifested. Mutation in the *ASXL1* gene was identified in 9/26 patients, however, no mutation in the *CBL* gene was detected. Distribution of observed genetic and genomic lesions in individual CML patients is shown in the Figure 1. Mutation T315I clustered with advanced phases, while other changes seem to be distributed randomly. **Conclusions.** Patients with primary or acquired resistance to TKIs showed different pattern of gene mutations and genomic aberrations. TKI resistance in CML patients is probably not associated with alterations in the p53 pathway. Additional changes potentially influencing treatment response were found. However, most important event facilitating disease progression is likely the presence of T315I mutation in *BCR-ABL1*, as this mutation was identified in almost all patients that progressed to advanced phases.This work was supported with CZ.1.05/1.1.00/02.0068, CZ.1.07/2.3.00/20.0045, CZ.1.07/2.4.00/17.0042, MSM0021622430, and MUNI/A/0784/2011.

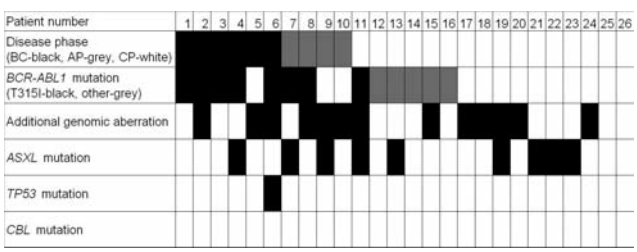

Figure 1. Distribution of genetic and genomic alterations in TKI-resistant CML patients.

0184

ETV6/ABL1 FUSION IN CHRONIC MYELOID LEUKEMIA

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Background. Chronic myeloid leukemia (CML) patients usually present with the Philadelphia (Ph) chromosome that results from t(9;22)(q34;q22) or variants, giving rise to a BCR/ABL1 fusion gene. Occasionally ABL1 forms chimeric products with 6 other genes. Of these, the ETV6/ABL1 fusion gene is the most common and has been reported not only in CML patients, but also in other haematological malignancies. **Aims.** To analyze the formation of the fusion gene ETV6/ABL1 in a female CML patient with t(9;12)(q34;p13) as sole bone marrow chromosome abnormality at presentation.METHODS and CASE REPORT: The BM G banding analysis failed to detect a Ph chromosome in a sample from a 42-year-old female who presented with CML. Instead a major cell population was found with a karyotype of 46,XX,t(9;12)(q34;p13) [8/10]. FISH using commercial probes did not show ABL1 changes (D-FISH/BCR/ABL1, Vysis), but revealed rearrangement of the ETV6 (ETV6/BA, Vysis). Whole genome screening was performed by a customized oligonucleotide x400K array (*Agilent Technologies*) and RT-PCR to search for rearrangements of ABL1 and ETV6 genes. A range of BAC probes was applied to obtain a comprehensive FISH map of the t(9;12)(q34;p13) products. **Results.** FISH showed rearranged ETV6 with 9q34 being the translocation partner. RT-PCR using published primers (*Van Limbergen et al., Genes, Chrom & Cancer 30:274-282, 2001*) confirmed the presence of ETV6/ABL1 both type A and B transcripts. However, such transcripts are unlikely to result from a direct rearrangement via t(9;12)(q34;p13) because of the contra orientation of the ETV6 and ABL1 genes. It is has been previously suggested that other genome changes affecting either of the partners are required before the formation of this fusion. In our case the genome array analysis provided a clue. The genome screening carried out with oligonucleotide arrays covering primarily exonic areas of over 2,000 genes revealed two main facts: (i) deletions within ETV6 consistent with rearrangements but unexpectedly, gains and losses within the NOTCH gene at 9q34.3 and (ii) a high level of genome instability evidenced by multiple cryptic imbalances affecting regions that harbor genes with known role in myeloid proliferation such as JAK2 (9p24.1), SYK (9q22.2), CCND1 (11q13.3), NTRK3(15q25.3) and RUNX1 (21q22.12) among others. We 'walked' with BAC probes along the 9q34 region and identified the breakpoint within NOTCH. Taken together, the array and FISH mapping data suggests a multistage mechanism for the formation of the ETV6/ABL1 fusion: firstly, translocation after breaks within NOTCH1 at 9q34 and within ETV6 at 12p13, followed by an inversion within the der(9) chromosome that results in formation of the ABL1/ETV6 fusion.SUMMARY: ETV6/ABL1 is a rare fusion gene in hematological malignancies, associated high genome aberration load and poor prognosis. We show that the formation of this rearrangement involves a third partner, the NOTCH1 gene and multiple cryptic genome lesions. These findings are reviewed in the context of 23 other similar cases published so far.

0185

EXPLORING THE MECHANISMS UNDERLYING RESPONSE VARIATION TO IMATINIB MESYLATE TREATMENT AMONG MALAYSIAN CHRONIC MYELOID LEUKEMIA PATIENTS - A MOLECULAR, EPIGENETIC AND PHARMACOGENETIC APPROACH

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Background. Despite the successful introduction of Imatinib Mesylate (IM) in Chronic Myeloid Leukemia (CML) treatment, success of this agent has been hampered by issues of clinical resistance and variability in treatment response. Various mechanisms for interindividual response variation have been described involving BCR-ABL1 dependent and BCR-ABL independent pathways. BCR-ABL dependent mechanism usually involves point mutations in the Tyrosine Kinase Domain (TKD) and amplification of BCR-ABL gene. BCR-ABL independent pathway still remains unclear with several postulated mechanisms. The HOX gene network encodes master regulators in haematopoiesis and

DNA methylation has been implicated to have an important role in aberrant control of HOX gene expression. Methylation of HOXA4 and HOXA5 has been strongly associated with progression to blast crisis and poor response to IM in CML patients. **Aims.** We hypothesized that DNA methylation of HOXA4 and HOXA5 might be associated with IM response variation. ABCB1 and ABCG2 are genes involved in IM transport and it was hypothesized that SNPs in ABCB1 and ABCG2 genes could also influence the efficacy of absorption or elimination of IM and modulate response variation. We explored the contribution of TKD mutations of BCR-ABL gene, methylation status of HOXA4 and HOXA5 genes, and SNPs of ABCB1 (T1236C, G2677T/A, C3435T) and ABCG2 (G34A, C421A) genes in affecting variation in response to IM in Malaysian CML patients. **Methods.** We employed dHPLC followed by sequencing for mutation analysis in 44 IM resistant CML patients, MS-HRM for methylation profiling and PCR-RFLP for genotyping of the 5 SNPs in 72 CML patients involving both IM good response and resistant CML patients. **Results.** Point mutations in the BCR-ABL were detected in 10/44 patients (22.7%). Six different types of mutations consisting of T315I(5), E255K(1), Y253H(1), M351T(1), V289F(1), E251G(1) were detected with T315I as the most predominant mutation. Point mutations in BCR-ABL TKD appeared to be a dominant BCR-ABL dependent mechanism accounting for resistance. Regarding BCR-ABL independent mechanism, HOXA5 was hypermethylated in both good response and resistant CML patients whereas HOXA4 methylation was higher in IM resistant (n=34) than IM good response CML patients (n=23) but the difference was insignificant (p=0.077). Some of the genetic variations of ABCB1 and ABCG2 correlated with response to IM. The genotype frequency of ABCB1 T1236T was significantly higher among good responders (p=0.039) whereas ABCB1 C1236C was higher among resistant group (p=0.0006). Likewise, ABCG2 C421C was significantly higher among resistant groups (p=0.004) but ABCG2 C421A was significantly higher among good responders (p=0.034). Other genotypes showed no significant difference between the two groups. **Summary and Conclusions.** Even though some of these SNPs influence IM response, our data are not significantly conclusive to translate into individual drug dose adjustment. The effect of IM depends on several genes and hence larger studies involving multiple genes exploring the potential effects of gene-gene interactions are clearly needed to elucidate the real impact of candidate gene polymorphisms and methylation profile on IM response and to what extent the use of second generation TKIs may eventually overcome the resistance imposed by genetic and epigenetic alterations.

0186

CLINICAL RELEVANCE IN CHRONIC MYELOID LEUKEMIA OF DELETION AND INSERTION EVENTS IN TYROSINE KYNASE DOMAIN OF BCR-ABL

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Background. CML is characterized by the t(9;22)(q34;q11) chromosomal translocation, which leads to the constitutively activated BCR-ABL tyrosine-kinase (TK). Current treatment of CML is tyrosine-kinase inhibitor (TKI) therapy: Imatinib, Dasatinib and Nilotinib. Although most of the patients achieve complete cytogenetic and major molecular responses, the others get a suboptimal response or may show a TKI resistance. The main cause is the appearance of BCR-ABL TK point mutations, which impair and interfere with drug binding. Patients showing a TKI-specific mutation are eligible to alternative TKI treatment. Rare cases of splicing events inducing deletion or insertion of multiple nucleotides into ABL TK have been describe. **Aims.** We characterized the ABL deletions/insertions in 24 CML patients that showed Imatinib resistance. **Methods.** A total of 830 patients, treated in front-line therapy with Imatinib, were monitored by RQ-PCR, according to ELN recommendations. In 225 suboptimal or failure patients the ABL sequence of Bcr-Abl gene was analyzed by DHPLC and Sequencing. Deletions/insertions of ABL were confirmed by ARMS-PCR. Based on wild type BCR-ABL crystallography, mutated sequences were analyzed by homology modeling both to generate 3D structures and to predict TKI bindings. Informed consent was obtained from all patients. **Results.** We detected ABL mutations in 118 out of 225 (52%) tested patients: 80% of them reported single aminoacid substitutions in ABL, whereas the remaining patients show ex7-9 ABL deletion (19%) or insertion (1%). Among this 20% of patients no differences were observed in terms of age (median 52±18, range of 26-78), sex and WBC count.We observed ABL D363-R386del in 4 patients and homology modeling reveals that the entire activation loop have been lost, without modification in Dasatinib H-bonds. Moreover, we identified some mutations do not modify TKI interactions in ABL, i.e. ABL t1143del causing a C-terminal truncated ABL protein; a simultaneously insertion of ABL 293-1P and Lys294Gln exchange. In this last case, the insertion seems to attenuate the Imatinib-bond power, probably due the steric hindrance by proline. Indeed, the ins293-1P clone became predominant during Imatinib therapy, whereas it disappears during Dasatinib treatment. Homology modeling showed that 15 deletion of 177-336 nucleotides and one insertion of 1 nucleotide events caused frameshift and early truncation of ABL with loss of 3 out 5 Imatinib and all two Dasatinib H-bonds. Among these 15 patients, 7 switched to Dasatinib and died of advanced phases, while the remaining patients continued Imatinib or switched to Nilotinib and are still alive in cytogenetic complete response. Two patients with a complex BCR-ABL rearrangement (ex4-9 ABL deletion and ex13 BCR insertion) lost the mutated clone during Imatinib treatment. **Conclusions.** The TK domain is significantly altered in the majority of the detected ABL deletion/insertion alterations and Ph+ mutated cells are prone to add further alterations giving survival advantages to the leukemic clone. In conclusion, screening for deletion/insertion ABL mutation associated with homology modeling analysis to predict TKI binding, may be an experimental tool to help tailoring therapy for patients with CML.

0187

INTERMITTENT IMATINIB (INTERIM) TREATMENT IN PH+-CML ELDERLY PATIENTS IN STABLE COMPLETE CYTOGENETIC RESPONSE

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Background. The introduction of Imatinib has significantly improved the outcome for patients with Ph+ CML. According to current recommendations, Imatinib (IM) should be continued indefinitely. However, optimal responders can be eligible for investigational trials of treatment discontinuation or modification. **Aims.** This study (ClinicalTrials.gov NCT 00858806) describes the effects of a policy of intermittent Imatinib (INTERIM) treatment (one month on/one month off) in patients ≥ 65 years old with Ph+ CML and stable complete cytogenetic response (CCgR), previously achieved with at least 2 years of standard (continuous) imatinib treatment. The primary endpoint of the study was the proportion of patients who maintained CCgR after 1 year of INTERIM (study core). **Methods.** Cytogentic and molecular responses were monitored by FISH and RT-Q-PCR every 3 months. Chromosome Banding Analysis (CBA) of marrow metaphases was performed at baseline, then only in case of FISH positivity (BCR-ABL positive nuclei > 1%) to define the loss of CCgR. The maximum tolerable rate of CCgR loss was statistically eastblished to 5%. **Results.** Seventy-six patients were enrolled, and were treated and monitored for a minimum of 12 months (study core). The clinical course was uneventful, in particular, no patient progressed to AP or BP, or lost complete hematologic response (CHR). No patient asked to resume the continuous treatment. At the end of the study-core (12 months), 6 patients (8%) lost CCgR (CBA-positive) and the probability of maintaining CBA-negativity was 92% (95% CI 86-98%). None of the patients who lost CCgR developed ACA in Ph+ cells. None of the factors which were examined by univariate and multivariate analysis was found to be asso-

ciated with CBA data, with the exception of the duration of imatinib therapy (HR=0.23, 95% CI 0.008-0.73, p=0.01). All the six patients who lost CCgR (CBA-positive) resumed continuous imatinib treatment at the same dose and returned in CCgR (CBA negative) after 3 to 9 months.At baseline, all patients but one (99%) were in MMR (MR 3.0), and 63 patients (83%) were in CMR (MR 4.0). At the end of the study core, these percentages were reduced to 76% and 58%, respectively. The probability of maintaining CMR (MR 4.0) was 63% (95% CI 52-75%) and MMR (MR3.0) was 76% (95% CI 67-86%). The percentage of patients in less than MMR (MR3.0) increased from 1% to 22% but no BCR-ABL mutations was found. None of the factors which were examined for a relationship with molecular response loss was found to be significant. After 12 months, the remaining 70 patients were allowed either to resume continuous treatment or to continue the intermittent treatment. Fifty-seven (75%) patients continue on INTERIM after 24 months, all in MMR (MR3.0) or in CMR (MR4.0). **Conclusions.** In summary, INTERIM produced a reversible loss of CCgR in 8% of patients but caused an increase of residual molecular disease in many more patients. However, INTERIM did not induce early a greater risk of disease progression and resistance to imatinib. **Acknowledgments.** EuropeanLeukemiaNet through the European Treatment and Outcome Study (EUTOS) and by Cofin 2009.

0188

ENEST1ST: NILOTINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): A EUROPEAN AND EUTOS CLINICAL INITIATIVE FOR STANDARDIZATION OF MOLECULAR RESPONSE

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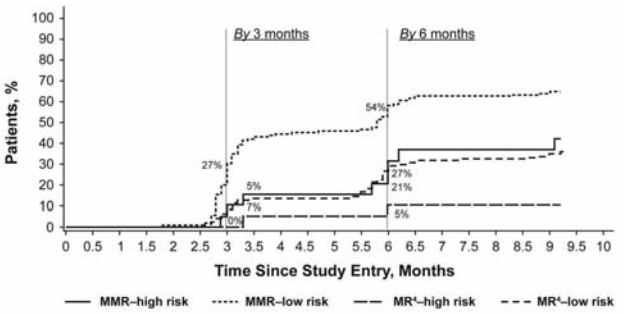
Background. Nilotinib, a potent selective inhibitor of BCR-ABL, was approved for the treatment of patients with newly diagnosed Ph+ CML-CP on the basis of data from ENESTnd. **Aims.** This phase IIIb, open-label study evaluated the efficacy and safety of nilotinib 300 mg BID as first-line therapy in patients with newly diagnosed Ph+ CML-CP. The primary endpoint was the rate of molecular response (MR^4,defined as detectable disease at ≤ 0.01% according to the international scale [IS] or undetectable disease in cDNA with ≥ 10,000 ABL transcripts) at 18 months as assessed in 13 European Treatment and Outcomes Study (EUTOS) laboratories to improve the sensitivity and standardization of RT-PCR for low-level BCR-ABL transcript detection. Secondary endpoints included MMR and CCyR at 12 and 24 months and rate of adverse events after achievement of MR^4. **Methods.** Patients with Ph+ CML-CP were administered nilotinib 300 mg BID. The MR^4 rate, defined above, was measured in peripheral blood leukocytes by quantitative RT-PCR, in 13 EUTOS laboratories. This is the first interim analysis of 200 patients who have reached 6 months. **Results.** To date, 1046 patients were recruited at 438 sites in 26 countries. 206 patients with at least 6 months follow up are presented here. Overall, 34%, 37%, and 18% of patients were classified as low, intermediate, and high Sokal risk; 80% and 9% of patients were classified as low and high EUTOS risk; remaining patients did not have available risk scores. In 191 patients with evaluable molecular samples, the CCyR rates by 3 and 6 months were 28% and 72%, and the MMR rates by 3 and 6 months were 22% and 52%, respectively. The MR^4 rates were 5% by 3 months and 19% by 6 months. Patients with low EUTOS risk had higher rates of both MMR and MR^4 by 3 and 6 months (Figure). Overall, 28% of patients had prior imatinib therapy (65% had > 6 weeks but < 3 months as allowed by protocol), and molecular responses were similar regardless of prior imatinib exposure. By 6 months, no patient had progressed to AP/BC. Adverse events (AE) were mostly grade 1-2 and manageable with dose interruptions/reduction: rash (22%), pruritus (17%), alopecia (12%), fatigue (12%), headache, and nausea (10%). Grade 3-4 hematologic AEs were uncommon. Grade 3-4 biochemical laboratory abnormalities included lipase increase (2%) and hyperbilirubinemia (2%). Treatment-related serious AEs were reported in 9% of patients. At 6 months, 89% of patients remained on study; 8% discontinued due to AEs. **Summary and Conclusions.** Current data demonstrate efficacy and safety of nilotinib 300 mg BID similar to that reported in other frontline trials, including ENESTnd. Through methodological improvements of RT-PCR, a network of EUTOS laboratories defined and measured MR^4 in a standardized manner. These efforts may further define deep molecular response to help identify patients potentially eligible for tyrosine kinase inhibitor discontinuation in future studies.



*n = 191 treated patients with evaluable molecular response data and typical transcripts.
EUTOS, European Treatment and Outcomes Study; MMR, major molecular response; MR^4, molecular remission with a sensitivity of 4 logs.

Figure 1. Molecular response by 3 and 6 months basd on EUTOS risk score.*

0189

FREQUENT AND SUSTAINED DRUG-FREE REMISSION IN THE AUSTRALASIAN CML8 TRIAL OF IMATINIB WITHDRAWAL

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Background. Selected patients with chronic myeloid leukaemia (CML) in stable complete molecular remission (CMR) on imatinib are able to stop treatment and may remain in remission. The prospective ALLG CML8 trial completed its accrual of 40 patients in August 2011. **Aims.** To identify risk factors for relapse when imatinib is stopped. **Methods.** Patients receiving imatinib for chronic phase CML were eligible if they were in CMR (no detectable BCR-ABL mRNA using real-time quantitative RT-PCR (RQ-PCR) with a sensitivity of at least 4.5-log) for ≥2 years. After giving informed consent patients stopped imatinib, and were monitored by RQ-PCR in a central laboratory: monthly for 12 months, 2-monthly in the second year, and 3-monthly thereafter. **Results.** 21 patients received imatinib after interferon therapy (IFN-IM cohort). 19 patients received imatinib frontline (IM cohort). The median follow-up was 33 months (IFN-IM 36mo; IM 33mo). 22 patients have met the study definition of relapse (BCR-ABL detected by RQ-PCR in two consecutive tests), while 18 patients remain in stable CMR after 5-66mo (median 33mo). Most relapses occurred within 6mo of stopping imatinib (median 3mo), but 7 relapses occurred later (range 6-24mo). Notably, late relapses generally did not show a rapid, exponential rise in BCR-ABL, whereas early relapses did. Imatinib treatment was recommenced at confirmed molecular relapse: 21/22 patients regained CMR, and the remaining patient was in MMR at last follow-up (14mo). No patient developed a kinase domain mutation. The actuarial molecular relapse-free survival was 42% (95% confidence interval 25-57%) at 33mo. This was similar between cohorts (IFN-IM 50%; IM 32%). We examined potential predictive factors for relapse (Table 1). Continuous variables were dichotomised about the median. In univariable analysis high risk Sokal score was associated with increased relapse risk (LO-INT 10/20; UNKNOWN 7/ 15; HI 5/5), as was a shorter duration of IFN treatment (in IFN-IM patients). Highly sensitive (6.2-log) and patient-specific BCR-ABL DNA PCR was used to monitor the level of minimal residual disease (MRD) in a subset of 25 patients with suitable diagnostic DNA available. 19/25 patients in CMR had detectable BCR-ABL DNA prior to stopping imatinib. Surprisingly, MRD detected by BCR-ABL DNA was **not** predictive of relapse risk, since 8 of these 19 patients remained in stable CMR with follow-up of 8-66mo. In stable CMR BCR-ABL DNA was detectable either intermittently or at a stable low level, around 1-log below the detection limit of RQ-PCR. With pro-

longed follow-up every patient had detectable MRD at least once while in CMR. No patient has become persistently DNA-negative so far. **Conclusions.** This study demonstrates that patients in stable CMR on imatinib can safely stop treatment with close molecular monitoring, and around 40% of these patients will remain in a durable CMR. These results are similar to the French STIM study, and are an important independent confirmation of the safety of this approach. DNA PCR studies suggest that the level of MRD is not the sole determinant of relapse risk. Further study is required to understand the biology of drug-free CMR.

Table 1. Potential predictive factors for molecular relapse.

Risk factor	No.	Relapse-free survival		P-value
Age	40	≤60 y	>60 y	
		52.5%	31.8%	NS
Sex	40	M	F	
		52.1%	34.3%	NS
Sokal score	25	Low-int	High	
		42.1%	0%	<0.001
Imatinib duration	38	≤70 m	>70 m	
		44.0%	42.1%	NS
IFN duration	21	≤12 m	>12 m	
(IFN-IM)		20.0%	59.6%	0.045
Baseline DNA PCR	25	Neg	Pos	
		53.6%	37.5%	NS

0190

EFFICACY AND SAFETY OF DASATINIB VS IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): EUROPEAN SUBPOPULATION ANALYSIS OF THE PHASE 3 DASISION TRIAL

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Background. In the phase 3 DASISION trial of dasatinib vs imatinib in patients with newly diagnosed CML-CP, dasatinib demonstrated higher 12-month rates of complete cytogenetic response (CCyR) and major molecular response (MMR), lower rates of transformation, and was well tolerated (Kantarjian NEJM 2010 362 2260). **Aims.** Assess efficacy and safety of dasatinib vs imatinib in European patients from DASISION. **Methods.** After informed consent, patients were randomized to dasatinib 100 mg once daily (n=259) or imatinib 400 mg once daily (n=260). 170 patients enrolled were from European countries (France: n=41; Spain: n=30; Czech Republic: n=22; Poland: n=20; Italy: n=14; Germany n=12; Belgium: n=9; Hungary: n=6; The Netherlands: n=6; Austria: n=4; Denmark: n=4; Greece: n=2), with 74 and 96 patients receiving dasatinib and imatinib, respectively. Primary endpoint was confirmed CCyR (cCCyR) by 12 months. **Results.** In the European subpopulation and total population, 82% vs 75% and 77% vs 66% of dasatinib vs imatinib patients achieved cCCyR by 12 months, respectively. After 24 months, 73% and 72% of European patients and 77% and 75% of total patients receiving dasatinib and imatinib, respectively, remained on therapy. Respective 24-month cumulative rates of MMR in the European subpopulation and total population for dasatinib vs imatinib were 61% vs 48% and 64% vs 46%, respectively. Median time to MMR calculated using competing risk analysis in European patients was shorter for dasatinib (12 months) than imatinib (30 months). Among European patients, no patient receiving dasatinib transformed to accelerated/blast phase (AP/BP) on study or during follow-up after discontinuation, compared with four (4.2%) patients receiving imatinib. In the total population, nine (3.5%) vs 15 (5.8%) patients receiving dasatinib vs imatinib transformed. Drug-related adverse events (AEs) are shown (Table 1). Grade 3/4 non-hematologic AE rates were ≤1%. For dasatinib vs imatinib, in the European population (total population), 43% vs 29% (59% vs 43%) had dose interruption, 22% vs 16% (28% vs 15%) had dose reduction, and 11% vs 6% (7% vs 5%) discontinued due to AEs. Minimum 36-month follow-up will be presented. **Conclusions.** After 24 months dasatinib showed higher efficacy compared with imatinib, lower transformations to AP/BP, and was generally well tolerated. The efficacy and safety of dasatinib in the European subpopulation was similar to the total population, supporting first-line dasatinib use in European patients with newly diagnosed CML-CP.

Table 1. *myalgia, muscle spasms, and musculoskeletal pain.

All-grade nonhematologic and grade 3/4 hematologic AEs in ≥10% of patients				
	Treated patients, %			
	Total population		European subpopulation	
	Dasatinib N=258	Imatinib N=258	Dasatinib N=74	Imatinib N=96
Nonhematologic (all grades)				
Fluid retention	25	43	19	49
Superficial Edema	11	36	8	47
Pleural Effusion	14	0	10	0
Myalgia*	22	39	19	46
Nausea	10	23	8	21
Diarrhea	19	21	14	24
Vomiting	5	11	1	7
Rash	11	17	8	16
Headache	13	11	7	15
Fatigue	9	11	12	18
Hematologic (grade 3/4)				
Neutropenia	24	21	14	12
Thrombocytopenia	20	11	7	6
Anemia	11	8	1	2

0191

THE HIGH RATE OF BLAST CRISIS IN IMATINIB TREATED CHRONIC MYELOID LEUKAEMIA WITH HIGH CIP2A LEVELS IS NULLIFIED BY FIRST LINE DASATINIB OR NILOTINIB.

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Background. We have shown that a high CIP2A protein level at diagnosis of chronic myeloid leukaemia (CML) is a prospective biomarker of the development of blast crisis (Lucas et al Blood 2011; 117: 6660). CIP2A acts by inhibiting PP2A (a phosphatase and tumour suppressor which regulates cell proliferation, differentiation and survival) resulting in stabilisation of both PIM1 and c-Myc, predisposing patients to blast crisis. However the effect of second generation tyrosine kinase inhibitors (2G TKI) in high CIP2A patients is unknown. **Aims.** To investigate the progression rate in patients with a high CIP2A protein level at diagnosis, when treated with the 2G TKI dasatinib or nilotinib from initial diagnosis. **Methods.** CIP2A, PP2A, pY^{307}-PP2A, PIM1 and c-Myc proteins were assessed by flow cytometry in 59 newly diagnosed chronic phase patients, of which 31 patients received imatinib and 18 received dasatinib or nilotinib as first line treatment. Patients were stratified according to their CIP2A protein level at diagnosis. High CIP2A patients were defined as those patients with a CIP2A protein MFI >7 (Lucas et al Blood 2011; 117: 6660). **Results.** Of the 18 patients who received a 2G TKI from original diagnosis, all have achieved complete cytogenetic remission (CCR). All 11 of these who had a high CIP2A level at original diagnosis are alive and have not progressed, with a median follow up of 31 months; in stark contrast to the 100% actuarial progression rate in patients with high CIP2A treated with imatinib (Figure 1).

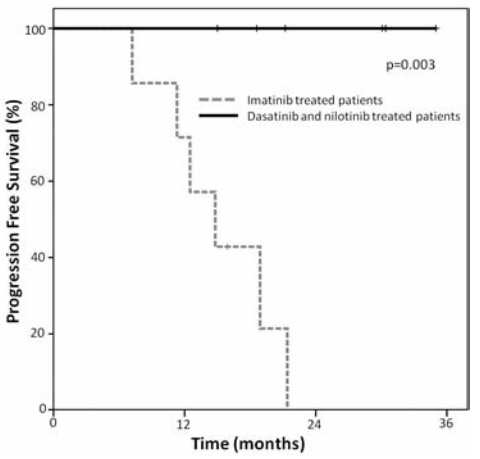

Figure 1.

High CIP2A patients treated with a 2G TKI had a trend towards a slower time to achieve MMR, compared to low CIP2A patients. At latest follow-up, no high CIP2A patient receiving a 2G TKI has yet achieved molecular negativity at the 4 log level (MR4.0), compared to 58% of low CIP2A patients (p=0.025).In K562 cells treated *in vitro* with TKI's at concentrations corresponding to Cmax, both dasatinib and nilotinib decrease CIP2A levels. In clinical samples, CIP2A protein level decreases in patients treated with either 2G TKI, and this is accompanied by an increase in PP2A activity and importantly a decrease in c-Myc serine 62 phosphorylation, thereby reducing its oncogenic activity. **Conclusions.** Patients presenting with high CIP2A levels have a very high risk of progression to blast crisis if treated with imatinib, but this risk is removed by using a 2G TKI as first line therapy. However, high CIP2A patients treated with 2G TKI have a lower probability of achieving deep molecular responses than those with low CIP2A levels, at least in the first 31 months of follow-up, and may merit more frequent observation. While these findings need independent confirmation, we hesitate to recommend imatinib for patients with high CIP2A protein levels at diagnosis unless very closely monitored.

0192

MOLECULAR RESPONSE KINETICS AND BCR-ABL REDUCTIONS IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) RECEIVING DASATINIB VS IMATINIB: DASISION 3-YEAR FOLLOW-UP

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Background. In the randomized phase 3 DASISION trial of dasatinib vs imatinib in patients with newly diagnosed CML-CP, dasatinib showed higher 12-month rates of complete cytogenetic response (CCyR) and major molecular response (MMR), lower transformation rates, and acceptable tolerability (Kantarjian NEJM 2010 362 2260). Recent reports indicate molecular responses of ≤10% BCR-ABL levels after three months of imatinib therapy are associated with increased overall survival and progression-free survival (PFS), and lower risk of failure (Hanfstein Blood 2010 116 abstract 360 and 2011 118 abstract 783; Marin JCO 2012 30 232). **Aims.** Analyze BCR-ABL kinetics in DASISION. **Methods.** After informed consent, patients received dasatinib 100 mg once daily (n=259) or imatinib 400 mg once daily (n=260). Typical BCR-ABL transcripts (b2a2 and b3a2) in peripheral blood were assessed using real-time quantitative PCR by a central independent laboratory. MMR was defined as BCR-ABL ≤0.1% on the international scale (IS; ≥3-log reduction from the standardized baseline). For PCR negative samples, ≥4.5 log sensitivity was confirmed (ABL level ≥25,614/reaction volume cDNA). **Results.** 24-month cumulative response rates in all patients calculated by competing risk analysis were higher for dasatinib vs imatinib: MMR: 65% vs 47%, P<0.0001; MR^4 ($BCR\text{-}ABL \leq 0.01\%$, ≥4-log reduction): 29% vs 19%, P=0.0053; $MR^{4.5}$ ($BCR\text{-}ABL \leq 0.0032\%$, ≥4.5-log reduction): 17% vs 9%, P=0.0032. Fewer patients receiving dasatinib (3.5%) transformed to accelerated/blast phase (AP/BP) vs imatinib (5.8%), on study or after discontinuation. The advantage in MMR rates for dasatinib over imatinib was maintained between 12 months (47% vs 28%) and 24 months (65% vs 47%). The advantage for dasatinib over imatinib at deeper levels of response ($MR^{4.5}$) increased between 12 months (5% vs 3%) and 24 months (17% vs 9%). Median time to MMR in all patients calculated by competing risk analysis was shorter for dasatinib (15 months) vs imatinib (35 months). Equivalent median BCR-ABL levels were achieved six months earlier with dasatinib than imatinib (Table 1).

Table 1.

BCR-ABL Kinetics		
	Median BCR-ABL level (IS), %	
	Dasatinib	Imatinib
3 Months	1.26	5.51
6 Months	0.30	1.01
12 Months	0.13	0.39
18 Months	0.06	0.15
24 Months	0.04	0.09

At three months, 84% vs 64% of evaluable patients receiving dasatinib vs imatinib achieved ≤10% BCR-ABL levels. Compared with patients who had >10% BCR-ABL levels at three months, patients achieving ≤10% had lower probability of transformation to AP/BP (dasatinib: 1.5% vs 8.1%; imatinib: 2.6% vs 9.4%), higher probability of 24-month PFS (dasatinib: 97% vs 83%; imatinib: 96% vs 85%), and higher probability of MMR by 24 months (dasatinib: 76% vs 16%; imatinib: 66% vs 19%). In this exploratory analysis, baseline characteristics were generally similar for patients achieving ≤10% and >10% BCR-ABL levels at three months. Compared with patients achieving ≤10% BCR-ABL levels, patients with >10%, had trends for intermediate/high Hasford risk scores, a greater proportion of splenomegaly, a larger spleen size among patients with splenomegaly, and higher WBC counts. Minimum 36-month follow-up will be presented. **Conclusions.** First-line dasatinib results in deeper and faster molecular responses vs imatinib, which are sustained with longer follow-up. In this exploratory analysis, earlier and deeper molecular responses appear associated with lower transformation rates and better long-term outcomes.

0193

EUTOS SCORE IS PREDICTIVE FOR SURVIVAL AND OUTCOME IN PATIENTS WITH EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA TREATED WITH NILOTINIB-BASED REGIMENS

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Background. The outcome of Philadelphia positive (Ph+) chronic myeloid leukemia (CML) patients has been significantly improved by the introduction of tyrosine kinase inhibitors (TKIs). Nilotinib (NIL) is a 2nd generation TKI with superior efficacy to imatinib (IM) approved as frontline therapy of CML in many countries. Until recently, the prognosis of CML patients has been evaluated using prognostic scores developed in the chemotherapy and in the interferon era. A new scoring system based on the analysis of a large cohort of CML patients treated with IM in early chronic phase (ECP), has been proposed by the European Leukemia-Net (ELN); the 2 variables included are basophils percentage and spleen size (Hasford J et al. Blood 2011;118:686-692). **Aims.** To investigate the prognostic value of the EUTOS score in a cohort of ECP CML patients treated frontline with NIL-based regimens. **Methods.** The patients were enrolled in two phase 2 multicentric studies conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00481052 and NCT00769327) or were treated with NIL as initial treatment at the Bologna University Hospital. Definitions: complete cytogenetic response (CCgR) was defined as the absence of Ph+ metaphases over at least 20 metaphases examined by conventional banding analysis or <1% BCR-ABL+ nuclei over 200 nuclei examined by I-FISH; major molecular response (MMR) was defined as $BCR\text{-}ABL < 0{,}1\%^{IS}$; failures were defined according to 2009 ELN criteria; progression was defined as the transformation to accelerated or blastic phase; deaths, for any reason and at any time. All the calculations were performed according to the intention-to-treat principle. **Results.** 215 patients were included; the median age was 53 years (range 18-86). The median follow-up was 29 months (range: 18-43 months). The patient distribution according to the different scoring systems was as follows: EUTOS score 95% low and 5%; Sokal score 38% low, 44% intermediate and 18% high; Euro score 39% low, 56% intermediate and 5% high. The cumulative CCgR rate was 93%; the cumulative MMR rate was 89%; the failure-free survival was 90%; the progression-free survival was 93% and the overall survival 94%. Patients with low EUTOS score achieved a significantly higher cumulative rate of MMR than patients with a high EUTOS score (91% vs. 60%, p=0.01). Moreover, and more importantly (Figure 1), the patients with low EUTOS score had higher failure-free survival (91% vs. 70%, p=0.02), higher progression-free survival (94% vs. 80%, p=0.05) and higher overall survival

(95% vs. 79%, p=0.04) than the patients with a high EUTOS score. The Sokal score was able to predict differences in terms of MMR at any time and of failure-free survival, but not in terms of progression-free survival or overall survival. The Euro score failed to detect any response and outcome difference. **Conclusions.** The proportion of the patients who failed on NIL was small, reflecting the efficacy of the drug. In spite of that, the EUTOS score, developed from IM-treated patients, was able to identify this small group of patients, better than the Sokal and the EURO scores. **Acknowledgements.** European LeukemiaNet, COFIN,BolognaUniversity, BolognAIL

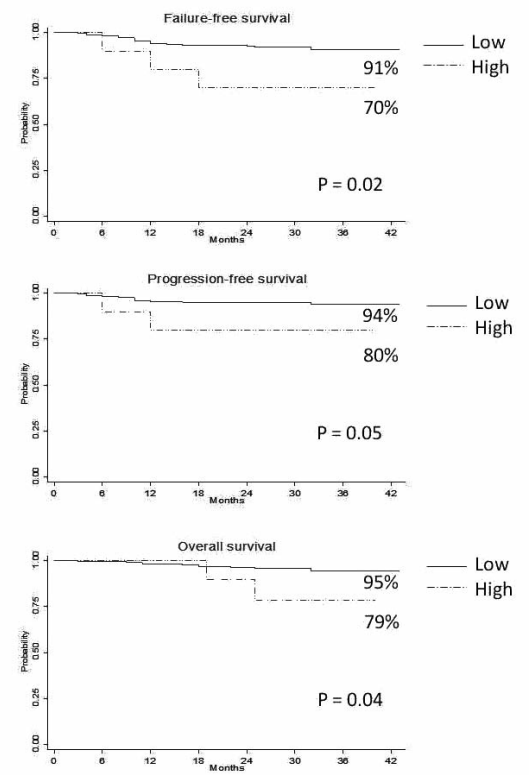

Figure 1. Outcome by EUTOS score.

0194

LOSS OF MAJOR MOLECULAR RESPONSE IS ACCURATE FOR RESTARTING IMATINIB AFTER IMATINIB DISCONTINUATION IN CP-CML PATIENTS WITH LONG LASTING CMR: IMPORTANCE OF FLUCTUATING VALUES OF MRD AND INTERFERON

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Background. Patients with fluctuating values of MRD have been observed after imatinib (IM) discontinuation in CML (Chronic Myelogenous Leukemia) patients (pts) in complete molecular response (CMR) for more than 2 years (STIM study, Mahon *et al*. Lancet Oncol. 2010). **Aims.** We analysed pts who stopped IM following a maintained CMR. Those patients were not included in the STIM study and were offered to restart therapy upon loss of major molecular response (MMR). We aimed to validate the loss of MMR as a criteria to restart IM. **Methods.** CP-CML pts were eligible if they were in CMR (CMR[4.5]: *BCR-ABL/ABL IS* <0.0032%) or UMRD (undetectable *BCR-ABL*) under IM therapy during 2 years. Some of these patients differed from the patients enrolled in the STIM study because they experienced one positivity of the *BCR-ABL/ABL* ratio during the 2 years follow-up. The criteria for restarting imatinib was the loss of MMR (BCR-ABL/ABL IS ratio >0.1%). We thus were able to calculate relapse free survival (RFS) using different criteria as end-points: loss CMR/UMRD defined by one occurrence MRD positivity, loss of CMR/UMRD using the STIM definition (two consecutive increasing values) and loss of MMR. **Results.** 58 CP-CML pts were included. Median follow-up after IM discontinuation was 17.2 months. Sex ratio (M/F) was 57% with a median age of 55.1 years. Sokal score distribution was 55.6%, 29.6% and 14.8% for low, intermediate and high risk respectively. 34 out of 58 (58.6%) pts received interferon therapy prior to IM. Median duration of IM therapy and median duration of CMR/UMRD prior to discontinuation was 77.2 months (30.1-145.4) and 37.1 months (24.7-96) respectively. Of note 31 out of 58 pts (53%) had a least one MRD positivity after the achievement of CMR/UMRD. We identified 18 patients (31%) who experienced repeated low levels of MRD after IM discontinuation without losing their MMR with a median follow-up of 23 months. We next analysed relapse free survival (RFS) using the loss of MMR criteria (RFS-MMR). Median RFS-MMR was not reached, compared to median RFS using the loss of CMR/UMRD criteria (4.8 months) and median RFS using the STIM criteria (13.7 months) (p=0.003). As a consequence, 65.6% of the patients remain treatment free at 2 years using the loss of MMR criteria. We then asked if prior therapy with interferon before IM may influence treatment free survival (TFS). Duration of IM therapy and Sokal score risk distribution were comparable in pts who received interferon or not. We observed a significantly longer TFS in interferon pre-treated pts as compared to patients who received IM first line (72% versus 55% at 2 years respectively, p=0.017). **Conclusions.** We validated the loss of MMR as robust criteria for restarting IM. We were able to identify up to 31% of pts in long term MMR with fluctuating values of BCR-ABL after IM discontinuation. We observed a better treatment free survival in patients previously treated with interferon before IM compared to patients who received IM as first line therapy emphasizing the putative role of interferon on CML stem cells.

0195

EARLY PREDICTORS OF PROGRESSION TO ACCELERATED-BLASTIC PHASE IN EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS TREATED FRONTLINE WITH NILOTINIB-BASED REGIMENS

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INTRODUCTION: Nilotinib is a second-generation tyrosine kinase inhibitor (TKI) that demonstrated higher and faster responses and lower progression rates compared to imatinib in early chronic phase chronic myeloid leukemia patients (ECP CML). Early cytogenetic and molecular responses have been associated to a better outcome for imatinib treated patients (Jabbour et al. Blood 2011; Marin et al. JCO 2011). Few data are available concerning the relationships between the rapidity of the responses and the probability of subsequent progression to accelerated-blastic phase (AP/BP) after nilotinib-based regimens frontline. **Aims.** to evaluate the rate of progression and to identify early predictors of progression in ECP CML patients treated with nilotinib-based regimens. **Methods.** Two hundred fifteen patients were enrolled in two multicenter phase 2 studies conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00481052 and NCT00769327) or were treated at the Department of Hematology and Oncological Sciences of S.Orsola-Malpighi Hospital - University of Bologna, with nilotinib 400 mg BID or 300 mg BID as initial treatment. The median age was 53 years (range 18-86). The median follow-up was 29 months (range: 18-43 months). Definitions: complete cytogenetic response (CCgR)

was defined as the absence of Ph+ metaphases over at least 20 bone marrow metaphases examined by conventional banding analysis or <1% BCR-ABL nuclei in peripheral blood by FISH (≥200 nuclei examined, I-FISH); molecular response was defined as the BCR-ABL/ABL ratio according to IS; progression was defined as the transformation to AB/BP. All the calculations were performed according to the intention-to-treat principle. **Results.** Overall, 8/215 (3,7%) patients progressed: 7/8 progressions occurred during the first year (2 patients at 4 months, 2 at 6 months, 2 at 10 months, 1 at 12 months and 1 at 13 months). At diagnosis 2 patients had clonal chromosome abnormalities; 2/8 patients had high EUTOS score, 6/8 intermediate (3) and high (3) Sokal score, 5/8 intermediate EURO score. At the time of progression the ABL mutational status was: 3 wild-type, 4 T315I, 1 Y253H. In order to identify early predictors of progression to AB/BP we analysed the cytogenetic and molecular response rates at 3 months in patients with and without subsequent progression. Seventy-four per cent (159/215) of the patients obtained a CCgR at 3 months, 3/159 progressed (1.9%); on the other hand, 26% (56/215) of the patients did not obtained a CCgR at 3 months (n=33) or were not evaluable (n=23), 5/56 (8.9%) progressed (p=0.0298)*. Eighty per cent (173/215) of the patients obtained a BCR-ABL/ABL ratio ≤ 1% at 3 months, 4/173 (2.3%) progressed; on the other hand, 20% (42/215) of the patients had a BCR-ABL/ABL ratio at 3 months > 1% (n=23) or were not evaluable (n=19), 4/42 (9.5%) progressed (p=0.0487)* **Conclusions.** In patients treated frontline with nilotinib-based regimens a CCgR and a BCR-ABL/ABL ratio ≤ 1% at 3 months correlated in univariate analysis with significantly reduced rates of progression to ABP. **Acknowledgements.** European LeukemiaNet, COFIN, Bologna University, and BolognAIL.

Table 1. Detail of patients that progressed to AP/BP. CCA: clonal cytogenetic abnormalities in the Ph+ clone; CgR: cytogenetic response; mCgR: minor CgR (Ph+ 36-65%); CCgR: complete CgR (Ph+ 0%); No CgR (Ph+ 100%); NE: not evaluable; WT: wild-type; INT.: intermediate

ID	CCA Baseline	SOKAL	EURO	EUTOS	Time to Prog. (Months)	CgR at 3 months	BCR-ABL/ABL ratio at 3 months	Mutation
1	YES	LOW	LOW	LOW	4	mCgR	0,5	T315I
2	NO	LOW	LOW	LOW	4	No CgR	11,1	T315I
3	NO	HIGH	INT.	LOW	6	CCgR	0,132	T315I
4	NO	INT.	INT.	HIGH	6	CCgR	2,82	Y253H
5	NO	INT.	INT.	LOW	10	NE	0,001	WT
6	NO	INT.	LOW	LOW	10	CCgR	0,03	WT
7	NO	HIGH	INT.	LOW	12	mCgR	13,4	WT
8	YES	HIGH	INT.	HIGH	13	NE	8,64	T315I

0196

EFFICACY AND SAFETY ANALYSIS OF SUBCUTANEOUS OMACETAXINE MEPESUCCINATE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA STRATIFIED BY RESISTANCE/INTOLERANCE TO AT LEAST 2 TYROSINE-KINASE INHIBITOR THERAPIES

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Background. Omacetaxine mepesuccinate ("omacetaxine") is an investigational, first-in-class cephalotaxine protein synthesis inhibitor that facilitates tumor cell death without depending on BCR-ABL signaling. Efficacy and safety were investigated in two phase 2, open-label, multicenter studies of patients with treatment-resistant chronic myeloid leukemia (CML) who had failed at least prior imatinib, many of whom were also resistant to or intolerant of dasatinib and/or nilotinib. **Aims.** This analysis of patients with CML and prior therapy with ≥2 tyrosine-kinase inhibitors (TKIs) assessed the efficacy and tolerability of omacetaxine across 3 groups: patients who had never achieved or had lost response to ≥2 TKIs (resistant group), were intolerant of ≥2 TKIs (intolerant group), or were resistant to 1 and intolerant of another (resistant/intolerant group). **Methods.** A subset of data from the phase 2 studies included patients in chronic phase (CP) and accelerated phase (AP) who were resistant/intolerant to ≥2 approved TKIs. All patients provided informed consent. Omacetaxine 1.25 mg/m^2 was given subcutaneously twice daily: ≤14 consecutive days/28-day cycle for induction, ≤7 days/cycle as maintenance. **Results.** Of 81 CP patients, 20% achieved major cytogenetic response (MCyR) for a median of 17.7 months (Table). Of these, 8 (10%) achieved complete cytogenetic response. Of 41 AP patients, 27% achieved major hematologic response (MaHR) for a median of 9 months (Table 1); none achieved a MCyR. Median cycles of exposure, study duration, and overall survival for each disease phase are also shown in the Table. Of 66 (81%) CP patients with treatment-related grade 3/4 adverse events (AEs; 54 resistant, 7 intolerant, 5 resistant/intolerant), the most common were thrombocytopenia in 44 resistant, 6 intolerant, and 4 resistant/intolerant patients and neutropenia in 32 resistant, 4 intolerant, and 1 resistant/intolerant patient. Fifteen CP patients had an AE leading to discontinuation (10 resistant, 2 intolerant, 3 resistant/intolerant), primarily disease progression. There were 10 deaths (the most common were disease progression and sepsis), 8 resistant, 1 intolerant, 1 resistant/intolerant; 2 were considered related to treatment (both sepsis). Of 28 AP (68%) patients with treatment-related grade 3/4 AEs (23 resistant, 3 intolerant, 2 resistant/intolerant), the most common were thrombocytopenia in 15 resistant, 2 intolerant, and 2 resistant/intolerant patients and anemia in 10 resistant, 3 intolerant, and 1 resistant/intolerant patients. Sixteen AP patients had an AE leading to discontinuation (16 resistant, 0 intolerant, 0 resistant/intolerant), primarily disease progression. There were 10 deaths due to AEs (the most common were disease progression and cerebral hemorrhage), 9 resistant, 1 resistant/intolerant; 2 were considered related to treatment (pancytopenia and febrile neutropenia). **Summary and Conclusions.** This subset analysis of patients with CML and prior therapy with ≥2 TKIs shows that omacetaxine provides efficacy and tolerability in both CP and AP across TKI-resistant, intolerant, and resistant/intolerant groups. Interpretation of the intolerant and the resistant/intolerant group data was limited by small sample sizes.Support - Teva Pharmaceutical Industries Ltd.

Table 1. Efficacy results.

Group	Resistant	Intolerant	Resistant +Intolerant	Total
Chronic Phase				
Mean age, 58 years	n=69	n=7	n=5	N=81
MCyR, n (%)	13 (19)	2 (29)	1 (20)	16 (20)
Median duration of MCyR, mo	Not reached	7.4	17.7	17.7
Median cycles	7	4	2	6
Median study participation, mo	9.1	7.3	2.3	8.8
Median survival, mo	33.9	Not reached	25.0	33.9
Accelerated Phase				
Mean age, 57 years	n=36	n=3	n=2	N=41
MaHR, n (%)	10 (28)	0	1 (50)	11 (27)
Median duration of MaHR, mo	11.2	Not reached	5.6	9.0
Median cycles	3	2	5	2
Median study participation, mo	3.5	3.0	5.8	3.4
Median survival, mo	16.0	Not reached	8.1	16.0

0197

SAFETY AND MANAGEMENT OF TOXICITIES IN THE PHASE 3 BELA TRIAL OF BOSUTINIB VERSUS IMATINIB IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

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Background. Bosutinib is an orally active, dual competitive Src/Abl kinase inhibitor. The BELA trial compared the safety and activity of bosutinib with imatinib in newly diagnosed chronic phase chronic myeloid leukemia (CP CML). **Aims.** This analysis summarizes the safety profile and toxicity management of each agent after ≥24 months of follow-up; updated data including ≥30 months of follow-up will be presented. **Methods.** Patients aged ≥18 years with newly diagnosed (≤6 months) CP CML were randomized to receive bosutinib 500

mg/day (n = 250) or imatinib 400 mg/day (n = 252). **Results.** Median age was 48 years (range, 19-91 years) for bosutinib and 47 years (range, 18-89 years) for imatinib (n = 252). Median treatment duration was 27.5 months for both arms, with 63% bosutinib patients and 71% imatinib patients still receiving therapy; 25% and 9% of patients discontinued treatment due to an adverse event (AE). Deaths occurred in 7 bosutinib patients versus 13 imatinib patients (including 1 and 2 CML-related deaths); of these, 2 and 4 deaths occurred within 28 days of treatment discontinuation. Bosutinib was associated with higher incidences versus imatinib of gastrointestinal toxicities (diarrhea [70% vs 25%], vomiting [32% vs 16%], upper abdominal pain [14% vs 7%]) and pyrexia (18% vs 12%), and lower incidences of edema (peripheral edema [4% vs 11%], periorbital edema [1% vs 15%]) and musculoskeletal events (myalgia [6% vs 12%], muscle cramps [4% vs 22%], bone pain [4% vs 10%]). Diarrhea (12% vs 1%, respectively) and elevated alanine aminotransferase (ALT; 18% vs 3%) were the most common non-hematologic grade 3/4 AEs.Diarrhea typically occurred during the initial month of treatment, with a median time to first event of 3.0 days on bosutinib (n = 173) and 43.0 days on imatinib (n = 62), and a median duration of an event of 3.0 days and 5.5 days, respectively. Diarrhea was managed with antidiarrheal medication (bosutinib, 69%; imatinib, 42%), temporary dose interruption (bosutinib, 23%; imatinib, 10%), and/or dose reduction (bosutinib, 8%; imatinib, 0%). Of the patients with a temporary dose interruption due to diarrhea, 36/39 bosutinib patients and 6/6 imatinib patients were rechallenged without recurrence of diarrhea or permanent treatment discontinuation due to diarrhea.Despite the higher incidence of ALT and aspartate aminotransferase (AST) elevations with bosutinib, most events occurred during the first 3 months of treatment and were transient (Table 1). AEs of ALT and AST elevation were managed with dose modification and/or concurrent medication, and the majority of events had resolved at the time of this analysis (Table 1). Eleven patients discontinued bosutinib and 1 patient discontinued imatinib due to ALT elevation; no patients discontinued due to AST elevation. No cases met Hy's Law criteria (ALT/AST ≥3xULN [upper limit of normal] and bilirubin ≥2xULN), led directly to hospitalization, or were associated with permanent hepatic injury or liver-related deaths. **Conclusions.** Bosutinib and imatinib had acceptable but distinct safety profiles in newly diagnosed CP CML. Bosutinib was mainly associated with gastrointestinal AEs and transient ALT/AST elevations that were managed with dose modification and/or concomitant medication.

Table 1.

	ALT elevation		AST elevation	
Parameter	Bosutinib	Imatinib	Bosutinib	Imatinib
No. patients who experienced an AE	79	21	66	22
Median time to first event, days	28.0	141.0	28.5	139.0
Median duration of an event, days	17.0	28.0	14.5	28.0
Event resolved, n (%)	70 (89)	19 (90)	57 (86)	18 (82)
Event management, n (%)				
Received dose reduction	27 (34)	2 (10)	11 (17)	2 (9)
Received dose interruption	44 (56)	5 (24)	29 (44)	5 (23)
No rechallenge	4 (9)	0	2 (7)	1 (20)
Rechallenge	40 (91)	5 (100)	27 (93)	4 (80)
Successful rechallenge[a]	32 (80)	4 (80)	27 (100)	4 (100)
Unsuccessful rechallenge	8 (20)	1 (20)	0	0
Received concurrent medication	23 (29)	6 (29)	15 (23)	5 (23)
Discontinued treatment due to event	11 (14)	1 (5)	0	0

[a]Successful rechallenge includes patients who did not experience subsequent ALT or AST AEs or experienced subsequent ALT or AST AEs that did not lead to treatment discontinuation.

0198

NILOTINIB 400 MG BID IN EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA: BEYOND 4 YEARS RESULTS REMAIN STABLE - THE GIMEMA CML WP TRIAL CML0307

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Background. Nilotinib is a potent and selective BCR-ABL inhibitor approved for the first line treatment of CML. The ENESTnd trial (24 months follow-up) showed higher and faster molecular responses and lower rates of progression to accelerated-blastic phase of nilotinib vs. imatinib. With imatinib 400mg (IRIS trial) the rate of events and progression to AB/BP were higher during the first 3-4 years. Consequently, a confirmation of the durability of responses to nilotinib after 4 years is extremely relevant. **Aims.** To evaluate the long-term outcome of patients treated with nilotinib 400mg BID as frontline therapy. **Methods.** A multicenter phase 2 trial was conducted by the GIMEMA CML WP (ClinicalTrials.gov.NCT00481052). Four years minimum follow-up data for all patients will be presented; data cut off for this analysis was December 31st 2011. Definitions: $MR^{3.0}$ (Major Molecular Response) as a BCR-ABL/ABL ratio <0,1%IS; $MR^{4.0}$, undetectable transcript levels with ≥10,000 ABL transcripts; failures: according to the revised ELN recommendations; events: failures and treatment discontinuation for any reason. All the analysis has been made according to the intention-to-treat principle. **Results.** 73 patients enrolled: median age 51 years; 45% low, 41% intermediate and 14% high Sokal risk. The cumulative incidence of CCgR at 12 months was 100%. CCgR at each milestone: 96% and 92% at 12 and 24 months, respectively. The overall estimated probability of $MR^{3.0}$ was 99%, while the rates of $MR^{3.0}$ at 12 and 24 months were 85% and 82%, respectively. Two out of 73 patients never achieved a $MR^{3.0}$, 1 who progressed to ABP (see below) and 1 in stable and confirmed CCgR at 36 months. Only 3 patients had a confirmed loss of MMR due to low adherence (all 3 still on nilotinib, 1 patient re-obtained a MMR). The overall estimated probability of $MR^{4.0}$ was 79%, while the rates of $MR^{4.0}$ at 12, 24 and 36 months were 12%, 27% and 30%, respectively. One third (22/73 pts) showed a stable $MR^{4.0}$ (defined based on 3 consecutive $MR^{4.0}$ samples 4 months apart). Only one patient progressed at 6 months to AP/BP and subsequently died (high Sokal risk, T315I mutation). The last daily dose was 600 mg or higher for 49 patients (67%). Six patients discontinued permanently nilotinib: 1 patient progressed to AP/BP; 3 patients had recurrent episodes of amylase and/or lipase increase (no pancreatitis); 1 patient had atrial fibrillation (unrelated to study drug) and 1 patient died after 32 months of mental deterioration and starvation (unrelated to study drug). During the fourth year of therapy (median follow-up of 45 months) no new events occurred; the estimated probability of overall survival, progression-free survival and failure-free survival was 97%, the estimated probability of event-free survival was 91%. **Conclusions.** Given the very low rate of failure and the stable molecular responses observed the outcome is expected to be optimal for most of patients after 4 years of nilotinib. **Acknowledgements.** European LeukemiaNet, COFIN, Bologna University, BolognAIL

0199

SIX-YEAR FOLLOW-UP OF PATIENTS WITH IMATINIB-RESISTANT OR IMATINIB-INTOLERANT CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) RECEIVING DASATINIB

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Background. Dasatinib, a potent BCR-ABL tyrosine kinase inhibitor (TKI), demonstrated significant efficacy in patients with imatinib-resistant and -intolerant CP-CML and is an approved second-line therapy. Recommended dosing is 100 mg once daily (QD), based on the CA180-034 dose-optimization study. Six-year analysis of CA180-034 provides the longest follow-up of patients with CP-CML treated with a second-line TKI. **Aims.** Investigate long-term safety and efficacy of dasatinib in patients with CP-CML resistant or intolerant to imatinib. **Methods.** Study design and endpoints have been described (Shah 2008, JCO). After informed consent, patients (N=670) were randomized to dasatinib 100 mg QD (n=167), 50 mg twice daily (BID; n=167), 140 mg QD (n=168), or 70 mg BID (n=168). **Results.** Results are based on five-year data; minimum six-year follow-up will be presented. After five years minimum follow-up, 205 (31%) patients remain on study drug (71% resistant to imatinib and 29% imatinib-intolerant). In the 100 mg QD arm, 57 patients (35%) remain on treatment (41 imatinib-resistant and 16 imatinib-intolerant). Patients still receiving study drug in the remaining treatment arms are as follows: 50 mg BID: n=52 (31%); 140 mg QD: n=44 (27%); 70 mg BID: n=52 (31%). 5-year efficacy results are presented (Table 1). In a landmark analysis, 85% and 83% of patients achieving major molecular response (MMR) and complete cytogenetic response (CCyR) at six months, and 85% and 79% of patients achieving MMR and CCyR at 12 months were alive and progression-free at five years, respectively. This represents a better outcome compared to patients with no cytogenetic response at six and 12 months (31% and 35% were alive and progression-free at five-years). For 100 mg QD, grade 3/4 hematologic adverse events (AEs) generally first occurred within 12 months of treatment and all-grade non-hematologic AEs generally first occurred within 24 months of treatment. Five-year rates of non-hematologic AEs (all-grades) for 100 mg QD vs other treatment arms include headache (33% vs 28%), diarrhea (28% vs 31%), fatigue (26% vs 23%), and pleural effusion (24% vs 32%). Dose/schedule modifications were permitted to manage AEs. Analysis of dosing by last dose available demonstrates that, across all arms, 151 (74%) patients were on QD dosing, of which 85 (56%) were receiving ≥100 mg QD. Association of long-term outcome and pre-therapeutic factors (age, best response to imatinib, baseline mutations, highest imatinib dose) will be presented. **Conclusions.** The majority of patients receiving dasatinib after five years continue on QD dosing. Long-term follow-up of dasatinib 100 mg QD demonstrates durable efficacy and a generally well-tolerated safety profile for patients with CP-CML following prior imatinib therapy.

Table 1.

Five-year efficacy rates								
	100 mg QD (N=167)		140 mg QD (N=167)		50 mg BID (N=168)		70 mg BID (N=168)	
	Imatinib resistant (n=124)	Imatinib intolerant (n=43)	Imatinib resistant (n=123)	Imatinib intolerant (n=44)	Imatinib resistant (n=124)	Imatinib intolerant (n=44)	Imatinib resistant (n=126)	Imatinib intolerant (n=42)
Major molecular response	41.7%	52.5%	32.7%	75.0%	36.1%	58.5%	39.5%	56.8%
Progression free survival	56. 2%	62.7%	39.9%	82.9%	63.7%	75.3%	55.8%	79.5%
Overall survival	76.7%	81.8%	75.5%	87.6%	72.7%	82.4%	69.9%	81.4%
Progression to advanced disease	5.6%	2.3%	4.1%	6.8%	6.5%	4.5%	5.6%	2.4%

0200

BCR-ABL KINETICS AFTER DISCONTINUATION OF IMATINIB IN CML PATIENTS WITH MR4.5 OR UNDETECTABLE MOLECULAR RESIDUAL DISEASE

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Background. Approximately 50% of chronic phase (CP) chronic myeloid leukemia (CML) patients who received Imatinib (IM) achieve complete molecular response (CMR) at 6-7 years. The recent data from a study showed that in patients with a CMR lasting at least 2 years, the probability of persistent CMR at 12 months was 41%, and suggested IM can be safely discontinued, at least in some patients with sustained CMR. However, to define whether discontinuation of IM can be safely employed, further validation and much longer follow-up are needed. **Aims.** This prospective study is performed to identify safer and more concrete indicators of successful discontinuation and explore contributing factors for sustained undetectable transcript. **Methods.** In our prospective, multicenter study, CP CML patients who were treated with IM for more than 3 years in sustained $MR^{4.5}$ or undetectable transcript for at least 2 years were enrolled. Our objectives were to evaluate the probability of persistent undetectable molecular residual disease (UMRD), $MR^{4.5}$, and major molecular response (MMR) at 12 months after discontinuation and to identify contributing factors for sustained MMR. After discontinuation, molecular response was monitored using RQ-PCR assay every month up to 6 month, every 2 months up to 12 month, and every 3 months thereafter. The loss of MMR, MR4.5, and UMRD were defined on 2 consecutive assessments. **Results.** Up to 14 Feb 2012, 32 patients (15 men and 17 women) were enrolled in this study. With a median age of 37.5 years (range, 19 - 74), the percentages of patients with low, intermediate and high Sokal risk scores were 28%, 28% and 13%, respectively with unknown Sokal risk scores in 31%. There were previous histories of allogeneic stem cell transplantation (SCT) in 12 patients and interferon (IFN) therapy in 3 patients, while 19 patients received first-line IM. The median time on IM therapy and the median duration of sustained CMR were 84 months (range, 40 - 113) and 54 months (range, 24 - 105), respectively, prior to discontinuation.After a median follow-up of 12.9 months since discontinuation of IM, loss of MMR was observed in 4 patients after a median time of 3.7 months (range, 2.9 - 3.8) of treatment discontinuation. Loss of $MR^{4.5}$ and UMRD were detected in 7 patients and 8 patients, respectively. The 12-month probability of sustained MMR, $MR^{4.5}$ and UMRD were 83.2%, 71.7%, and 69.1%, respectively. By the univariate analysis, previous SCT, the time from IM therapy to UMRD, and the UMRD duration before discontinuation of IM were potential factors on sustained MMR. Among 20 patients without previous SCT, the time from IM therapy to UMRD and the UMRD duration before discontinuation of IM were potential predictive factors.**Conclusions** Our data showed a higher 12-month probability of sustained UMRD compare with previous discontinuation studies. Overall, of the 12 patients with previous SCT, no patient lost MMR. Through subgroup analyses in the cohort excluding previous SCT recipients, we found that the time from IM therapy to UMRD and the UMRD duration before discontinuation of IM were potential predictive factors.

Hodgkin's lymphoma

0201

DIMINISHING PROGNOSTIC ROLE OF PREEXISTING DIABETES MELLITUS FOR PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA IN THE RITUXIMAB ERA

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Background. Rituximab reforms the treatment of diffuse large B cell lymphoma (DLBCL) and the prognostic significance of baseline patient features should be reevaluated. Few population-based studies have investigated the association of diabetes mellitus (DM) and outcomes of lymphoma; however, the results remain inconclusive. **Aims.** To delineate the prognostic role of DM in DLBCL **Materials and Methods**. From January 2000 to December 2009, a total of 468 consecutive newly-diagnosed DLBCL patients receiving first-line chemotherapy with CHOP or rituximab plus CHOP (R-CHOP) were enrolled. Preexisting DM was defined according to medical history, use of anti-diabetic medications, or any record of an abnormal hemoglobin A1c test. Progression-free survival (PFS) and overall survival (OS) were estimated and compared using the Kaplan-Meier method with a log-rank test. **Results.** CHOP was administered in 194 patients, and 274 patients received R-CHOP. DM was identified in 16.2% (76/468) of patients. Diabetic patients were older and more performance-restricted, compared to the non-DM patients in both the CHOP and R-CHOP groups. In the CHOP group, 5-year PFS and OS were inferior in DM patients (PFS 32.4% vs. 50.0%, p = .045; OS 38.2% vs. 62.5%, p = .008). However, outcomes were similar for both DM and non-DM patients in the context of R-CHOP treatment (PFS 69.0% vs. 57.3%, p = .104; OS 78.6% vs. 70.7%, p = .197). The response rate of chemotherapy in DM patients was also improved to a level similar to non-DM patients with rituximab use. **Conclusions.** The prognostic significance of preexisting DM in DLBCL patients is changing in the rituximab era. The potentially additional benefit of rituximab in DM patients merits further investigation.

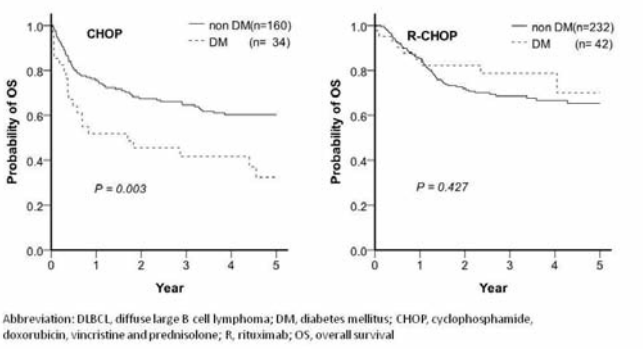

Figure 1. Overall survival of DLBCL patients with or without DM under CHOP/R-CHOP.

0202

DOMINANCE OF NAÏVE AND CENTRAL MEMORY CD4+ T CELLS IN THE CLASSICAL HODGKIN LYMPHOMA MICROENVIRONMENT PROVIDES A DISTINCTIVE SIGNATURE WITH IMPLICATIONS FOR PATHOGENESIS AND CLINICAL DIAGNOSIS

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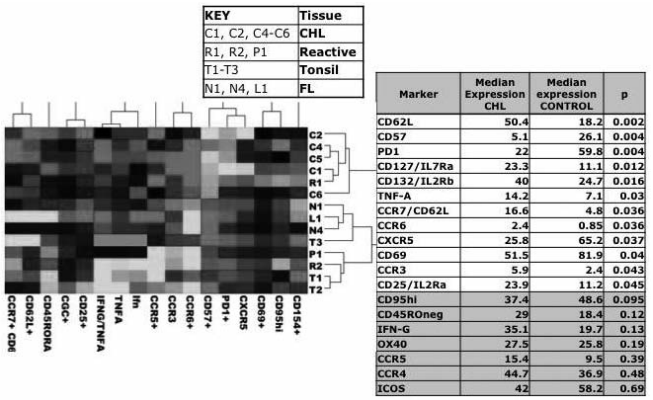
Background. The microenvironment of classical Hodgkin lymphoma (CHL) is dominated by CD4+ T cells. The functional interactions of this infiltrate with the malignant Hodgkin-Reed-Sternberg (HRS) cell and other microenvironment components including macrophages and FOXP3-expressing Tregs have prognostic and pathophysiological significance. **Aims.** Using CD4+ T cell-expressed markers of known or proposed functional importance in CHL, we characterized dominant markers at diagnosis, identifying those which discriminate CHL-infiltrating from benign or other germinal centre B cell malignant node-infiltrating CD4+ T cells and hence determine a CHL-specific CD4+ T cell phenotype to functionally and clinically validate. **Method**. Frozen single cell suspensions (SCSs) from diagnostic lymph node biopsies (CHL=5, FL=3, tonsil=4, reactive=3) were thawed and immunofluorescent staining was performed for CD3/CD4 along with 42 markers chosen for their functional importance in CHL (immunoglobulin/tumour-necrosis-factor superfamily members, chemokine receptors, memory, TH2, TH1, T-Follicular-Helper (TFH)-associated markers, activation, senescence, immunosuppressive mediators and cytokines with TH1, TH2, immunosuppressive and HRS stimulatory properties (Figure 1). Flow cytometry was performed, markers expressed as a % total CD3+CD4+ population and medians calculated for each tissue (CHL vs. FL vs. tonsil vs. benign). T tests determined significant differences between CHL and other tissue-derived cells. Additionally, expression levels for each sample was entered into unsupervised hierarchical cluster analysis applying Pearson correlation/complete linkage. **Results.** Markers of central memory (CD62L, CCR7 and IL7Ra/CD127), naivety (CD45RO-neg), RANTES-receptor CCR3 and TH1-associated CCR5 (as well as IFN-G and TNF-alpha) and IL2 receptor components (CD132 and CD25) were overexpressed in CHL-derived CD3+CD4+ cells compared to controls, but there was no evidence of TH2 cytokines or IL10 expression. Markers of early activation (CD69, CD95), chronic activation/senescence (CD57&PD1) and TFH cells (CXCR5&PD1) were underexpressed. Treg/TH2-marker and CHL-associated TARC-receptor CCR4 was heterogeneously expressed in CHL (15-75%) as were receptors for HRS-expressed TNFRSF members (OX40: 25-60% and ICOS: 30-75%) but median expression was not significantly greater than in any control nodes. Unsupervised hierarchical clustering analysis identified two groups, one containing all CHL samples, the other FL and benign samples suggesting that the extended phenotype of the CD3+CD4+ compartment alone is characteristic of CHL. **Conclusions**. CHL-infiltrating CD3+CD4+ cells appear distinct from those infiltrating other reactive and malignant nodes with a phenotype suggesting overrepresentation of markers of central memory and naivety, and absence of early inflammatory or senescence markers, discriminating its phenotype from other malignant or benign node-derived CD3+CD4+ cells. As expected, expression of TARC-receptor CCR4 is increased but not in association with TH2-defining cytokines nor at levels significantly different to other malignant and benign reactive nodes. This dominant component suggests an expansion of the interfollicular T cell zone by central memory and naïve T cells perhaps through passive attraction into CHL-involved nodes, providing an explanation for the cell mediated immune defect in CHL, and a potentially diagnostic CD3+CD4+ signature for further clinical validation.



Marker	Median Expression CHL	Median expression CONTROL	p
CD62L	50.4	18.2	0.002
CD57	5.1	26.1	0.004
PD1	22	59.8	0.004
CD127/IL7Ra	23.3	11.1	0.012
CD132/IL2Rb	40	24.7	0.016
TNF-A	14.2	7.1	0.03
CCR7/CD62L	16.6	4.8	0.036
CCR6	2.4	0.85	0.036
CXCR5	25.8	65.2	0.037
CD69	51.5	81.9	0.04
CCR3	5.9	2.4	0.043
CD25/IL2Ra	23.9	11.2	0.045
CD95hi	37.4	48.6	0.095
CD45ROneg	29	18.4	0.12
IFN-G	35.1	19.7	0.13
OX40	27.5	25.8	0.19
CCR5	15.4	9.5	0.39
CCR4	44.7	36.9	0.48
ICOS	42	58.2	0.69

Figure 1. CHL lymph node-infiltrating CD4+ T cell phenotype is distinct from that of other benign and malignant lymph nodes.

0203

PERSISTENCE OF CD30 EXPRESSION IN CD30-POSITIVE LYMPHOMAS FOLLOWING TREATMENT WITH BRENTUXIMAB VEDOTIN (SGN-35)

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Background. Hodgkin Lymphoma (HL) and systemic Anaplastic Large Cell Lymphoma (sALCL) are two lymphomas that express CD30 on their cell surface. CD30 is a member of the tumor necrosis factor receptor superfamily. Brentuximab vedotin (SGN-35), an antibody-drug conjugate, consists of microtubule disrupting agent monomethyl auristatin E (MMAE) attached to the CD30-specific antibody, cAC10, by a protease cleavable dipeptide linker. The effect of brentuximab vedotin exposure on CD30 expression in patients undergoing treatment and post treatment are unknown. To examine the impact of brentuximab vedotin on CD30 expression, we performed a retrospective analysis of patients (pts) with HL and sALCL who received brentuximab vedotin at City of

Hope National Medical Center (COH), then subsequently underwent tumor biopsies upon disease progression. **Methods.** Between October 2009 and July 2011, 8 pts with relapsed/refractory HL or sALCL received brentuximab vedotin IV every 3 weeks at COH. Following disease progression, tumor biopsies were obtained. CD30 immuno-staining was performed using the monoclonal mouse anti-human CD 30, clone Ber-H2 (No M 0751), and visualized with the DAKO Envision/HRP kit. **Results.** While receiving treatment with brentuximab vedotin, 6 pts with HL and 2 with sALCL achieved disease control. The median age was 32 (range: 24-43). The median number of 3-week cycles of brentuximab vedotin was 6.5 (range: 2-10). 3 pts achieved a best response of CR, 4 patients achieved PR, and 1 pt achieved SD. 4 pts who had achieved a PR or SD had progressive disease while on brentuximab vedotin treatment. 4 additional pts who had achieved a CR or PR discontinued brentuximab vedotin treatment because of peripheral neuropathy (1), went onto allo-HCT (2), or changed salvage therapy to ICE while in PR (1). These 4 pts later developed progressive disease. All pts underwent biopsy of their lymphoma at the time of progression, 8/8 revealed ongoing CD30 expression. (Figure 1, IHC from 6 pts shown) **Conclusions.** These data suggest that relapsed or progressive HL expresses CD 30 even after prior exposure to brentuximab vedotin.

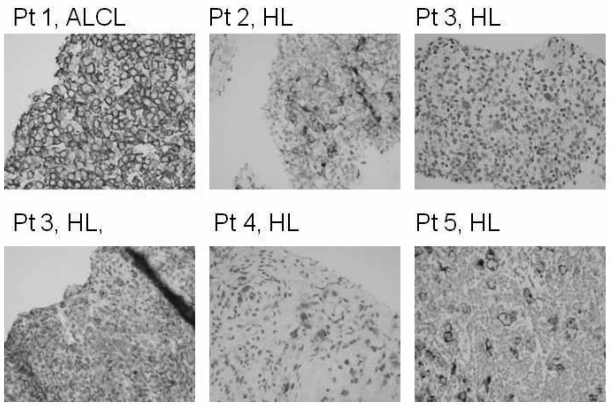

Figure 1.

0204

HEALTH STATE UTILITIES FOR RELAPSED/REFRACTORY (REL/REF) HODGKIN LYMPHOMA (HL) AND SYSTEMIC ANAPLASTIC LARGE-CELL LYMPHOMA (SALCL)

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[3]Oxford Outcomes Inc., San Francisco, United States of America

Background. There is a paucity of effective treatment options for individuals with rel/ref HL and sALCL. Recent advances in targeted therapies have shown promise in inducing disease remission. Due to increasing pressure on limited healthcare resources, many countries now seek to undertake a review of cost-effectiveness for newly developed therapies. Mechanisms for this evaluation process vary internationally but typically require the benefits of a treatment to be expressed in quality-adjusted life years (QALYs). This approach seeks to capture the value of a treatment in terms of change in both survival and quality of life (QOL). If formal assessment of QOL has not been undertaken in a trial, this provides a challenge in determining suitable data to support the evaluation process. **Aims.** The aim of this study was to capture QOL data for outcomes associated with receiving therapy for rel/ref HL and sALCL. These data were expressed as health state utility values ranging from 0 (dead) to 1 (full health) (Brazier et al, Eur J Health Econ 2011), suitable for supporting economic evaluation. **Methods.** Health state descriptions were developed depicting different rel/ref HL and sALCL treatment outcomes. These included descriptions associated with extent of disease symptoms (B-symptoms) and clinical response (complete or partial response, stable or progressed disease). Descriptions were based upon a review of published literature, discussions with five clinical experts, and five patient interviews. Once devised, the draft descriptions were reviewed by six experienced specialist clinicians to assess their clinical realism, accuracy, and degree of representativeness, before being piloted with six members of the general public. The health states were then valued by a representative sample of members of the UK (n=100) and Australian (AUS, n=75) general public using the time trade-off (TTO) task. This exercise elicits a utility value by examining an individual's willingness to trade-off years of life to avoid the consequences of living in a presented state. **Results.** The utility values elicited for the health state descriptions are presented in the table. Any treatment response was viewed by participants as being associated with a notable improvement in QOL (higher mean utility values), with the increase for partial response versus stable disease being +0.084 (UK) and +0.109 (AUS). The improvement suggested by the complete response utility value is particularly marked, representing a +0.196 (UK) and +0.224 (AUS) increase over stable disease. The presence of residual B-symptoms following treatment affected QOL, as evidenced by the lower mean utility value for stable disease with B-symptoms compared with stable disease without B-symptoms (difference of -0.124 for UK, and -0.110 for AUS). **Conclusions.** Societal valuation of health states for rel/ref HL and sALCL reveals the notable perceived benefit of a treatment response, while the experience of residual B-symptoms can be associated with a substantial decline in QOL. Health state utility values from the UK and Australia TTO valuations were broadly similar, with identical ordering in terms of preference (highest to lowest utility values).

Table 1. UK and Australian utility values for the health state descriptions.

Response	Complete	Partial	Stable	Stable (plus B-symptoms)	Progressed
UK data, mean (SD)	0.906 (0.08)	0.794 (0.17)	0.710 (0.20)	0.586 (0.27)	0.382 (0.28)
AUS data, mean (SD)	0.889 (0.16)	0.774 (0.22)	0.665 (0.26)	0.555 (0.32)	0.319 (0.31)

AUS, Australia; SD, standard deviation

0205

ACTIVITY OF BRENTUXIMAB VEDOTIN IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA OR SYSTEMIC ANAPLASTIC LARGE-CELL LYMPHOMA: COMPARISONS WITH META-ANALYSES OF HISTORICAL CHEMOTHERAPY DATA

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Background. Brentuximab vedotin (SGN-35) has been shown to have antitumor activity in terms of overall response rate and complete remission (CR) rate in two single-arm pivotal phase 2 trials in relapsed or refractory (rel/ref) Hodgkin lymphoma (HL; SG035-0003) and systemic anaplastic large-cell lymphoma (sALCL; SG035-0004). **Aims.** In the absence of suitable comparator regimens for these trials, and per the ICH E10 (CHMP/ICH/364/96) guideline, we conducted meta-analyses of historical chemotherapy data to compare the activity of brentuximab vedotin to other therapies used for treatment of rel/ref HL and sALCL. **Methods.** CR rate was the endpoint since achieving a CR may be predictive for long-term clinical benefit in HL and aggressive non-Hodgkin's lymphoma (NHL). For the rel/ref HL meta-analysis, PubMed searches identified prospective trials and retrospective case series in which ≥10 adult HL patients with rel/ref disease were treated with gemcitabine alone/in combination (one of the most commonly used systemic therapies beyond second-line treatment in HL). Trials were classified as post-autologous stem cell transplant (ASCT; ≥33% of enrolled patients), or ASCT-naïve. For the rel/ref sALCL meta-analysis, a PubMed search identified all trials since 2001 of aggressive rel/ref NHL that included at least one sALCL patient. Quantitative statistical meta-analyses were carried out per pre-specified plans, and performed using random effect models; estimates (95% CI) of overall CR rate were provided. **Results.** For the rel/ref HL meta-analysis, 16 trials (N=605 patients) were identified, including nine post-ASCT trials (n=296, median of three prior regimens) in which a median of 44% (range 15-64%) of patients had prior ASCT, and seven ASCT-naïve trials (n=309, median of one prior regimen). CR rates are shown in the figure. Estimated overall CR rate in the post-ASCT subgroup was 15% (95% CI: 6.5, 23.5), which was significantly lower than the CR rate of 34% (95% CI: 25.2, 44.4; p=0.003) seen with brentuximab vedotin in SG035-0003, in which all patients had prior ASCT and the median number of prior regimens was 3.5. In the ASCT-naïve subgroup, estimated overall CR rate was 35% (95% CI: 16.9, 52.2); however, these patients had less advanced disease and were less heavily pretreated. Considering all trials, estimated overall CR rate in patients with rel/ref HL was 24% (95% CI: 14.2, 33.9), with a trend towards

lower activity versus brentuximab vedotin in patients with post-ASCT rel/ref HL (p=0.148). For the rel/ref sALCL meta-analysis, 19 trials (N=752 NHL patients) were identified, which included 48 sALCL patients. The estimated overall CR rate, 18% (95% CI: 11.3, 24.5), was significantly lower than with brentuximab vedotin in patients with rel/ref sALCL (versus CR rate at time of meta-analysis: 53% [95% CI: 39.9, 66.7], p<0.0001; updated CR rate [ASH 2011]: 59% [95% CI: 45, 71]). **Conclusions.** The anti-tumor activity of brentuximab vedotin, in terms of CR rate, appears to exceed that of gemcitabine-based therapies in rel/ref post-ASCT HL patients, and also appears to exceed that of other treatment options for aggressive rel/ref NHL, including sALCL. These meta-analyses suggest that brentuximab vedotin may provide meaningful long-term clinical benefit in rel/ref HL and sALCL.

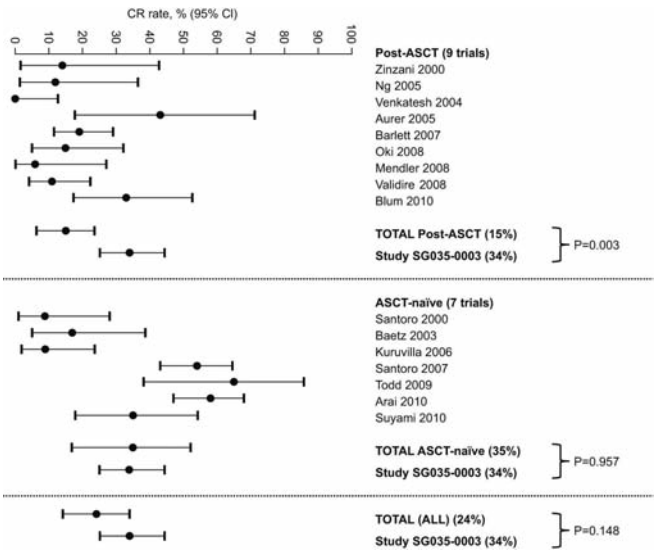

Figure 1.

0206

EFFICIENT HDAC INHIBITION AND TARC REDUCTION IN PATIENTS WITH REFRACTORY HODGKIN LYMPHOMA TREATED WITH RESMINOSTAT - PK/PD DATA FROM THE PHASE II SAPHIRE STUDY

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Background. Resminostat (R) is an oral pan-histone deacetylase (HDAC) inhibitor that has shown anti-tumor activity in a broad panel of preclinical models and revealed a favorable safety and efficacy profile in a first-in-man phase I study in patients (pts) with various solid tumor types. R is currently in phase II clinical development in pts with hepatocellular carcinoma (HCC), colorectal carcinoma (CRC) and Hodgkin lymphoma (HL). **Methods.** The open label, single arm, phase II SAPHIRE trial included HL patients who had progressed after prior therapy or were refractory to treatment. R was administered once daily at 600 mg or 800 mg. Pts were treated in cycles of 5 consecutive days followed by a 9 day treatment-free period (5+9 schedule), constituting a 14 day treatment cycle. Pts underwent assessment of their disease status by PET/CT. Primary endpoint of the study was the overall objective response rate (ORR). Secondary endpoints included efficacy, safety and tolerability and the analysis of pharmacokinetics of both doses for up to 6 h post-dose during the 1st and 3rd treatment cycle. Similarly, the effect of different doses of R on pharmacodynamic biomarkers such as HDAC enzyme inhibition and changes in expression levels of selected target genes was determined in peripheral blood cells (PBMC). In addition, plasma levels of the CC thymus and activation-related chemokine TARC (CCL17), a potential prognostic factor in HL, were determined. **Results.** The SAPHIRE study enrolled 37 patients of which 35 were eligible for efficacy evaluation. R at 600 mg or 800 mg daily dose was well tolerated with grade 2-3 anemia and thrombocytopenia in 35% and 27% of pts as most frequent AEs. Peak plasma levels of R were achieved at T_{max} = 2 h post-dose in both 600 mg (n=19) and 800 mg (n=18) dose cohorts. C_{max} and AUC increased proportionally to dose. Single and repeat PK profiles in both dose cohorts were comparable. Inhibition of HDAC enzymatic activity was time dependent and fully reversible within 72 h post-dose and reached a maximum of up to 94% inhibition at about 2 h post-dose corresponding to R peak plasma levels. Baseline TARC plasma levels of median 8400 pg/ml (range 35 pg/ml to >100 000 pg/ml) were substantially reduced after three cycles of R to a median of 2800 pg/ml, with a reduction of ³50% in 12 of 27 pts tested. Lower baseline TARC plasma levels were associated with clinical benefit to R treatment. Expression analysis of a set of 10 genes in pts PBMCs showed a correlation to drug plasma levels with strongest effects on transcription 5 h post-dose reflecting the delayed biological response to target inhibition. **Conclusions.** PK/PD data from the phase II SAPHIRE study indicate good bioavailability and efficient *in vivo* HDAC inhibition by resminostat at daily doses of 600 mg and 800 mg. Resminostat treatment also resulted in biological relevant downstream effects like transcriptional modulation and TARC plasma level reduction in heavily pre-treated HL patients. These findings may now be explored for their potential to predict clinical responses.

0207

SURVIVAL OF HODGKIN'S LYMPHOMA PATIENTS IN GERMANY

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Background. Hodgkin's lymphoma (HL) is a rare, highly treatable lymphoma. Previous publications have demonstrated high overall rates of survival for HL patients in Germany but no published information is available regarding survival by age, gender, or histologic subtype. **Aims.** To evaluate 5-year relative survival of patients with HL in Germany. **Methods.** Data was derived from 11 cancer databases throughout Germany, representing a total of about 33 million people. Patients diagnosed in Germany with HL (ICD 10 code C81) in 1997-2006 were included in the analyses. Period analysis was used to calculate 5-year relative survival rates for the time period of 2002-2006, overall and by gender, age, and histology. Similar calculations using data from the Surveillance, Epidemiology, and End Results database in the United States (US) were performed for comparison. A comparison of survival in 2002 versus 2006 was performed to evaluate recent changes in survival. **Results.** 5835 cases were identified, with a 9.15% of cases identified by death certificate only, leaving 5300 cases for analysis. Overall 5-year relative survival for patients with HL in Germany was 84.3%, compared with 80.6% in the US. Survival in Germany was highest in patients aged 15-29 at 97.9% and decreased with age to 57.5% at age 60+. Survival was about equal for men and women in Germany (84.7% in women versus 84.1% in men) but higher for women in the US (83.6% and 78.2%, respectively). Nodular lymphocyte predominant HL (NLPHL) had a higher survival than other types, with 5-year relative survival ranging from 72.2% for ages 60+ to 100.3% for ages 15-29. Survival was higher in Germany than in the US for all histologic sub-types except for NLPHL, with differences ranging from -0.9% units to +11.6% units. Survival improved in 2006 compared to 2002 for patients age 30-39, reaching almost 98% in 2006, and for patients with NLPHL, but was stable for other ages and histologies. Thus, in 2006, patients age 30-39 had a survival equal to patients age 15-29. **Conclusions.** Five year survival for patients diagnosed with HL in Germany is high, reaching nearly 98% for younger patients, but decreases with age. Survival did not significantly vary by gender in Germany but was worse for men in the US. These findings suggest that further concentration on treatment of HL in older patients and possibly a greater focus on how women are being treated may further improve survival for patients with HL.

0208

SAFETY AND EFFICACY OF BENDAMUSTINE THERAPY IN WALDENSTROM MACROGLOBULINEMIA

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Background and Aims. Waldenstrom Macroglobulinemia (WM) is a rare low-grade lymphoma characterized by the presence of lymphoplasmacytic cells in

the bone marrow and monoclonal IgM in peripheral blood. Bendamustine is a bifunctional alkylating agent that is approved for treatment of lymphocytic leukaemia and indolent non-Hodgkin's lymphomas. So far few data are available on its role in WM. Aim of the study was to test the efficacy and safety of Bendamustine +/- Rituximab therapy in untreated or relapsed WM afferred to our institution. **Methods.** A retrospective analysis was performed on 15 WM patients who received therapy with Bendamustine from June 2009 to November 2011 in a single Italian Centre. Eleven patients were male, median age was 74 years (58-81) and all patients had stage IV disease. Based on the Morel ISS-WM study, 3 (20%) patients were high risk, 4 (27%) were intermediate risk, and 8 (53%) were low risk. Six patients received Bendamustine at diagnosis, while 9 patients as second or subsequent lines of treatment (2 in second,3 in third, 4 patients >3 previous lines respectively). Bendamustine was administered at the standard dose of 90 mg/mq day 1-2 every 28 days in 11 patients, whereas four patients received a dose reduction of 70 mg/mq. Only two patients didn't receive Rituximab due to refractory disease. Median number of delivered cycles was 4 (2-6). **Results.** After a median follow up of 10 months (2-31), 13 patients were eligible for efficacy analysis (two patients are still on therapy). The ORR was 92%. We observed eleven (85%) partial response (PR), one complete response (CR) and one stable disease (SD). CR was obtained in one patient with low-risk ISS treated at diagnosis. We observed a reduction of M protein >50% in 11/13 (85%) patients, whereas in 7/13 (54%) we obtained a reduction of M protein >75%. Moreover, a reduction of >50% of adenopathies and splenomegaly was showed in 12/13 (92%) patients. The therapy with Bendamustine was well tolerated with a low incidence of adverse events, mainly of G1. Three patients developed a G1 or more neutropenia and one of them interrupted therapy for prolonged G4 neutropenia. Three patients were treated with G-CSF. Two pts developed G2 thrombocytopenia. No patients developed a ≥G3 thrombocytopenia. The most common non-haematological adverse events were G1 fatigue (one patient) and infections (two exacerbation of herpes zoster infection requiring treatment were described). **Conclusions.** In our retrospective analysis therapy with Bendamustine +/- Rituximab was well tolerated and effective for WM patients both at diagnosis and at progression. Based on these observations, larger prospective studies to evaluate the role of Bndamustine plus Rituximab in WM patients are warranted.

0209

DUAL-POINT FDG-PET: A PROMISING SCANNING TECHNIQUE IN HODGKIN LYMPHOMA

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Background. Interim ^{18}F-FDG-PET (iPET) is the most important prognosticator in advanced-stage ABVD-treated Hodgkin Lymphoma (HL), but in early stage with or without bulky lesion a low PPV of iPET was reported. Moreover, a single residual FDG-avid mass (SFAM), commonly found at treatment (Tx) end in HL, was shown to reduce the specificity of PET in Tx response evaluation. Dual-point PET scan (2P-PET) has been proposed to discriminate unspecific inflammatory from neoplastic FDG uptake. **Aims.** we report here preliminary results from a cohort of HL patients (p), scanned with 2P-PET with the aim to increase specificity and PPV. **Methods.** from December 2008 till January 2012 25 HL p from Italian, French and Polish centers, most of them with bulky lesions, underwent 2P-PET at baseline (2P-PET-0), after 2 ABVD (2P-PET-2) and at Tx end (2P-PET-end). 2P-PET scan consisted in 2 consecutive image acquisitions 60' (+60) and 120'(+120) after single, standard-dose FDG injection. Tx was ABVD (x4 or x6) or BEACOPP (4+4) ± consolidation/involved-field radiotherapy (RT). In 23/25 p no Tx change was done based on iPET results and in 2 according to protocol. Bulky was defined as a mass with largest diameter ≥5 cm. Scans were reviewed by 2 expert readers in a consensus session. Standardized-Uptake Value $(SUV)_{MAX}$ was calculated both in +60 and +120 using single or multiple Volume of Interest regions (VOIs) drawn to encompass a single or multiple spots of FDG uptake within a single or multiple nodal mass. Retention Index (RI) was defined as $[(SUV_{MAX}+120-SUV_{MAX}+60)/SUV_{MAX}+120]$%. In case of multiple spots inside a single node or within different nodes, only the single focus with FDG uptake increase was considered. **Results.** In 25 p (1 stage I,13 II, 4 III, 7 IV), 35 2P-PET were done. 10 p underwent 2P-PET-0: in 10/10 SUV_{MAX} increased from +60 to +120 (ΔSUV_{MAX} 1-4.3). 15 p had a 2P-PET-2: 12 with a mean-follow-up of 18.36 months were evaluable. 3 were excluded because of too short (+1, +5, +6 months) follow-up. 7/12 p showed an average RI reduction of 55% (200-14): all are in continuous CR (CCR). 5/12 p. showed an average 20% (11-26) RI increase: all progressed +1 to +9 months after Tx end. Overall 3/12 cases (1 false positive, 2 false negative, with a Deauville score of 4 and 2,3 respectively) were correctly classified after 2P-PET scan. Ten p with a SFAM had a 2P-PET-end: in the 6/10 with an increased average RI of 23% (9-43) a biopsy proved HL relapse. In 4/10 an average reduced RI of 29% (9-45) was found: in 2 biopsy disclosed presence of residual thymic along with inflammatory tissue and 2 were in CCR +4 to+10 months after Tx end. **Conclusions.** SUV_{MAX} increased in all patients scanned at baseline and at Tx end in relapsing HL, and decreased in patients remaining in CCR. This suggest that a mounting and dropping FDG avidity with time reflects presence of viable tumour cells and non-neoplastic tissue, respectively. The PPV and a PNV of 2P-PET were 100%.

0210

BRENTUXIMAB VEDOTIN IN 65 RELAPSED/REFRACTORY HODGKIN'S LYMPHOMA: PRELIMINARY REPORT OF A MULTICENTER ITALIAN RETROSPECTIVE STUDY

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Background. Hodgkin lymphoma (HL) is characterized by the presence of CD30-positive Hodgkin Reed-Sternberg cells. The antibody-drug conjugate brentuximab vedotin (BV) delivers the highly potent antimicrotubule agent monomethyl auristatin E to CD30-positive malignant cells. In a pivotal, phase 2, single-arm, multicenter study with BV the overall response rate (ORR) was 75% and the complete response (CR) rate was 34% (Chen et al, ASH 2010). Moving from these results eligible patients in Italy were granted early access trough a Named Patient Program (NPP). **Aims.** To evaluate the effectiveness and safety of BV as single agent in patients with relapsed/refractory HL treated under the NPP. Here we report preliminary results. **Methods.** A multicenter retrospective/prospective observational study is ongoing at 9 Italian hematology centers. Response to therapy was assessed by Revised Response Criteria for Malignant Lymphoma and any adverse events occurred during was recorded and classified for type, grade and relationship with BV. **Results.** The sample for this preliminary analysis consisted of 65 patients with median age at diagnosis of 27,5 years (range 12-71). Approximately half of the patients were male (52%). Eastern Cooperative Oncology Group performance status at baseline was 0 (37%) or 1 (40%) or 2 (23%). All patients but 8 had previously received an autologous stem cell transplant and the median number of prior chemotherapy regimens was 4 (range 2-13). More than 60% of the patients had primary refractory disease and 81% was also refractory to the most recent salvage therapy. At the last update, the median number of cycles was 6 (range 1-13). Consistent with findings from the pivotal phase 2 study, after the first evaluation (interim PET after 3 cycles, 60 evaluable patients) 12 (20%) patients had CR and 32 (53.3%) patients obtained a partial response (PR) with a global ORR of 73.3%; PET evaluation after 8 cycles (20 evaluable patients) showed 5 (25%) CR and 3 (15%) PR. To date, 9 patients were allowed to received an allogeneic stem cell transplant after treatment with BV. Grade 3-4 treatment-related adverse events (AEs) reported were neutropenia (9%), peripheral sensory neuropathy (9%), thrombocytopenia (8%) and hyperglycemia (2%). No related Grade 5 events were observed. **Conclusions.** BV was associated with manageable adverse events and, based on investigator assessment, demonstrated encouraging activity in heavily pretreated patients with relapsed or refractory HL. These preliminary results confirm the important tumor shrinkage, ORR and CR rate also in everyday clinical practice.

0211

EARLY HODGKIN LYMPHOMA THERAPY, BASED ON PREDEFINED RISK FACTORS AND EARLY INTERIM PET/CT. ISRAELI H2 PROTOCOL: PRELIMINARY REPORT

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Background. The current goal of treatment for Hodgkin lymphoma (HL) is to maximize response and minimize long-term toxicity. **Aims.** This study was aimed to prospectively evaluate the outcome of patients with HL whose therapy is chosen based on baseline prognostic factors and is tailored depending on the results of PET/CT imaging performed after 2 cycles of chemotherapy. **Methods.** In this multicenter ongoing study, initiated in 2006, we escalate the intensity of therapy for patients with suboptimal early response evidenced by PET/CT. Patients with classic HL aged 18-60 years, stages IA-IIA are eligible. Individuals with early disease stage are categorized according to the German Hodgkin Study Group criteria for early favorable and unfavorable HL. Following 2 cycles of ABVD (adriamycin, bleomycin, vinblastine, dacarbazine), patients with early favorable disease (EF) and a negative PET/CT undergo involved nodal radiation therapy (INRT 25.2Gy) and those with early unfavorable disease (EU) receive 2 more cycles of ABVD (a total of 4 cycles) followed by INRT(27.2-30Gy for bulky mediastinal mass). Patients whose interim PET/CT remains positive are given 2 additional cycles of ABVD (a total of 4-6 cycles), followed by radiation therapy. Patients with putative large irradiation field and interim negative PET/CT could have additional 2 X ABVD and radiation therapy could be spared. **Results.** One hundred and twenty three patients were enrolled, 113 had interim PET/CT. Eighteen patients had early favorable and 105 early unfavorable disease. Median patients' age was 33 years. Nine patients (two with EF and seven with EU HL) had disease progression; 2 of them despite augmentation of therapy and 7 patients who had interim negative PET/CT. The positive predictive value of interim positive PET/CT and negative predictive value of negative studies were 2/15(13%) and 89/96 (93%), respectively. Fifteen patients had escalation of therapy following positive interim PET/CT and 2 of them had disease progression. Ninety six patients with early disease had negative interim study and were treated with the minimal therapy considered to be safe. Eighty nine of them are yet in remission. After a short follow-up, (median of 23 months; range 7- 63), the current study appears to demonstrate a 3-year progression-free survival (PFS) of 92%. While the negative predictive value for the patients with negative interim study was 93%, the majority of relapsing patients had a negative interim PET/CT. **Conclusions.** The current preliminary results suggest that interim PET/CT has a low sensitivity, indicating that augmentation of therapy based on positive PET/CT should be mild in patients with HL stages IA-IIA. Further follow-up evaluating the late effect of INRT is required.

0212

COMPARING BRENTUXIMAB VEDOTIN OVERALL SURVIVAL DATA AGAINST PUBLISHED OUTCOME DATA IN PATIENTS WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA POST-AUTOLOGOUS STEM CELL TRANSPLANT (ASCT)

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Background. Treatment options for relapsed/refractory (rel/ref) Hodgkin lymphoma (HL) post-ASCT are limited. At this late stage life expectancy is short, and the few treatment options available are associated with high morbidity. Brentuximab vedotin represents the first therapy directed against the CD30 surface antigen expressed by HL cells and other haematologic malignancies. Brentuximab vedotin has been studied in HL patients who had relapsed following ASCT (SG035-0003; see Younes et al, ICML 2011, for response/safety data). This single-arm phase II trial formed the basis for the accelerated approval of brentuximab vedotin by the US FDA for HL patients post-ASCT or after ≥2 prior multi-agent chemotherapy regimens in non-ASCT patients. **Aims.** To compare the overall survival (OS) of rel/ref HL patients receiving brentuximab vedotin post-ASCT to OS associated with other treatments in this setting. **Methods.** A systematic review of English-language studies in rel/ref HL post-ASCT was carried out in Embase, Medline, Medline In-Process and CENTRAL from database inception until October 2010. ASH, EHA, ESMO and ASCO conference proceedings were also searched. More than 80% of the population in each study had to be HL patients who had failed ≥1 ASCT. No restrictions regarding study design or treatment were imposed. Treatments were broadly divided into two groups: second (mostly allogeneic) stem cell treatments (SCTs) and non-SCTs (radiotherapy, chemotherapy, palliative care). Where available, percentages of patients alive at six-monthly intervals for up to five years were extracted. Comparative graphs were produced, with proportions of patients alive in each study plotted against the time period of reporting, and each point sized to reflect number of patients per study, akin to a forest plot. **Results.** In total, 35 of the 49 studies retrieved by the systematic review reported OS. Of these, 31 provided retrievable data. *S*econd SCTs and non-SCTs performed similarly in years 1-2; however, a higher proportion of patients receiving second SCTs were alive in years 3-5 versus non-SCTs. Results varied substantially between studies; however, data from the largest studies, such as Martinez 2010 (second SCTs, n=133; non-SCTs, n=294) and Robinson 2008 (second SCTs, n=285), appeared representative, with data around the median of the observed range. At 1.5 years brentuximab vedotin showed promising outcomes, with an OS of 79% (n=102; data cut-off March 2011). This compares favourably with data from Martinez 2010 and Robinson 2008 for both second SCTs (1.5-year OS: 74% and 61%) and non-SCTs (1.5-year OS: 47%). Similar survival advantages were seen versus the remaining studies, except for two small non-SCTs studies (n=17, n=34) and three small second SCT studies (n=38, n=14, n=10). Producing a comparison between treatment types is hampered by the lack of randomised evidence and variation in patients' baseline characteristics, including exposure to further lines of systemic therapy post-ASCT. **Conclusions.** The 1.5-year OS for brentuximab vedotin of 79% is high relative to other published data for rel/ref HL patients post-ASCT. Further follow-up of SG035-0003 is required to determine if this improvement is maintained. Analyses adjusting for potential confounders would be required to robustly compare brentuximab vedotin to treatment options currently used.

0213

RETREATMENT WITH BRENTUXIMAB VEDOTIN IN CD30 POSITIVE HEMATOLOGIC MALIGNANCIES: A PHASE 2 STUDY

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Background. Brentuximab vedotin comprises an anti-CD30 antibody conjugated by a protease-cleavable linker to a microtubule-disrupting agent, MMAE. In pivotal phase 2 studies in patients with relapsed/refractory Hodgkin lymphoma (HL) or systemic anaplastic large cell lymphoma (sALCL), objective response rates were 75% and 86% and median durations of response were 6.7 and 12.6 months, respectively. **Aims.** A phase 2 study was initiated to investigate if patients who have previously responded to brentuximab vedotin could achieve another remission with retreatment (ClinicalTrials.gov #NCT00947856). **Methods.** Patients had a CD30-positive hematologic malignancy, achieved an objective response (per Cheson 2007) with prior brentuximab vedotin treatment, and experienced relapse after discontinuing treatment. Informed consent was obtained for all patients. Brentuximab vedotin was administered IV 1.8 mg/kg every 21 days; antitumor activity was assessed by the investigator. **Results.** 14 HL patients and 8 sALCL (5 ALK-negative) patients were enrolled (median age 34 years, range 16-72). Patients had received a median of 4 prior chemotherapy regimens (range 2-12). Median time since the previous brentuximab vedotin treatment was 6.9 months (range 1-44). Median number of retreatment cycles was 7 (range 1+ to 32+). Adverse events (AEs) in >25% of patients were nausea (41%), fatigue (36%), peripheral sensory neuropathy (36%), and diarrhea (27%). The most common Grade 3/4 AEs were anemia, fatigue, and hyperglycemia (3 patients each). Of the 11 patients who had pre-existing peripheral neuropathy, 3 (27%) had worsening with retreatment. Best clinical responses

in patients with HL were 3 CR, 5 PR, 3 SD, 3 PD. Among patients with sALCL, 5 achieved a CR, 1 had PD, and 2 were not yet evaluated. Of the 8 patients with CR in retreatment, previous best responses to brentuximab vedotin treatment were 4 PR and 4 CR. Median duration of retreatment response was 10.8 months (range 0+ to 10.8), and in patients who achieved CR, the median duration of response was not reached (range 0+ to 10.5 months); 11 patients remain on retreatment. **Conclusions.** Retreatment with brentuximab vedotin was generally well tolerated. Objective responses were observed (13 of 20; 65%) in this heavily pretreated population. Enrollment to the phase 2 retreatment study is ongoing.

0214

BENDAMUSTINE IN HEAVILY TREATED HODGKIN LYMPHOMA, A RETROSPECTIVE FRENCH STUDY IN 28 PATIENTS

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Background. Hodgkin lymphoma (HL) is a curable disease in up to 80% of patients but for the remaining relapsed or refractory (rel/ref) patients no standard salvage therapy exists. Thus new chemotherapeutic agents are needed for HL in relapse following autologous stem cell transplant (ASCT). Bendamustine is a bifunctional alkylating agent, recently FDA approved for the treatment of chronic lymphocytic leukemia and indolent B-cell non-Hodgkin lymphoma. Limited data supports the use of bendamustine in HL (Borchmann, et al. Ann Oncol 1998). **Aims.** This retrospective study evaluate the activity of single-agent bendamustine in rel/ref HL for ASCT failures, non myeloablative transplant (NMT) failures, or patients (pts) who are ineligible for transplant. Our primary outcomes are response rate, toxicity and, progression free survival (PFS) (calculated from the first dose to progression). **Patients and Methods.** From July 2009 to july 2011, 28 patients (16M/12F) with a median age of 25 years (18 -64y) received at least one dose of bendamustine in 7 french centers. First line chemotherapy was ABVD in 25 cases and 12 patients were primary refractory to ABVD and 7 patients received radiation therapy. The median number of previous chemotherapy lines was at 5 (4 - 8) and 4 patients never received ASCT because of no response, 24 received ASCT (tandem autotransplant in 9 cases) and 6 NMT before treatment with Bendamustine. Before the first dose of bendamustine 17/28 patients were "on"chemotherapy and all had platelet counts over 150 G/l. Bendamustine was given for 2 days at 120 mg/m² in 8 patients and 90 or 100 mg/m² in the remainings, it was associated with rituximab in4 pts, vinorelbine in one pt.RESULTS.Two patients received only one course of Bendamustine for grade 4 thrombopénia (bone marrow involvement in one pt) and died rapidly from progressive disease.7 ptsreceived 1-2 cycles,10 ptsreceived 3-4 cycles,9 ptsreceived 5-6 cycles and2 ptsreceived 8 & 12 cycles. 5 patients experienced at least one grade 4 thrombopenia and G-CSF was used in 5 patients.The maximal response (evaluated with PET in 13 cases) was CR/CRu in9 ptsand 6 PR with an overall response rate of 53%. The median PFS was at 8 months (range 1 -18+) and three patients received subsequent consolidation (REVLIMID n =1 Chlorambucil n = 1 & NMT n =1). At the stopping date(December 2011), 10 patients had died from the disease. **Conclusions.** Bendamustine is effective in this heavily pretreated group of patients (mostly end-stage HL) even if the PFS is short. An earlier use should be explored in rel/ref HL, most likely in combination with other drugs such as liposomal doxorubicine or brentuximab vedotine.

0215

CD5- MONOCLONAL B-CELL LYMPHOPROLIFERATION (MBL): CLINICAL CHARACTERISTICS AND OUTCOME

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Background. CD5- MBL is a rare and not very well described entity. Based on the absolute lymphocyte counts (ALC), it can be divided in low count CD5(-) MBL (with normal ALC) and clinical CD5(-) MBL (ALC>5000/μL). **Aims.** To further analyze the clinical and laboratory characteristics as well as the clinical course of CD5- MBL and to compare the 2 subcategories. **Methods.** Inclusion criteria were detection of CD5- monoclonal B-cell population in blood without any evidence of other organ involvement. Clinical and laboratory data was analyzed along and bone marrow was evaluated by morphology and immunohistochemistry , while FISH analysis was performed for detection of trisomy 3,18,12,del7q and del17p. All patients underwent whole body CT scanning and gastroscopy was performed in 20 patients. Informed consent has been obtained from all patients. **Results.** The studied population included 43 patients with a median age of 69 years. 21 of them had ALC<5000/μL and 22 presented with lymphocytosis. The clinical and laboratory characteristics are shown on table 1. No significant differences were noticed between the 2 groups besides paraproteinemia which was more common in low count MBL. Cytopenias and B-symptoms were absent. According to IPI most patients ranked in the low or low-intermediate risk group. Paraproteinemia was a common finding (20/43). Lymphoma cells presented moderate to strong expression of CD20, moderate expression of FMC-7, CD5 negativity while CD23 was positive in 12, CD11c in 14, CD25 in 6, SIgκ in 26 and SIgλ in 17 cases. Bone marrow was infiltrated in all patients and the pattern of infiltration was interstitial in 11, nodular in 6, intrasinusoidal in 7 and mixed in 13 (nodular and intrasinusoidal in 7, nodular and interstitial in 4, nodular and diffuse in 2) with no significant differences between the two subgroups. FISH analysis was performed in 8 pts and only in one of them deletion of 17p was detected. Among patients with low count MBL none of them required therapy at a median follow up of 27 months: 19 had stable CBC, 2 presented a gradual increase of ALC, while in one patient MBL regressed a year later. In the clinical MBL group at a median follow up of 48 months 10 pts had stable CBC, 9 had a gradual increase of ALC and 2 of them developed cytopenias 57 and 79 months after diagnosis respectively, while 1 patient developed splenomegaly and pancytopenia 130 months later. These 3 patients were treated with rituximab and achieved complete response. Furthermore, 2 patients had resolution of lymphocytosis 6 and 24 months after diagnosis respectively. 1/3 rituximab responders relapsed and died 117 months after diagnosis due to disease progression. **Conclusions.** CD5- MBL displays many similarities with marginal zone lymphomas. Patients with low counts MBL present an indolent natural course with excellent outcome, while clinical MBL seems to have a more aggressive clinical course and it may represent a distinct entity, as we have previously described with the term primary bone marrow marginal zone lymphoma.

Table 1.

	All pts	MBL <5000/μL	MBL >5000/μL
	43	22	21
Age	69 (38-85)	69 (53-77)	67 (38-85)
Male sex	17 (39%)	8 (36%)	9 (43%)
B-symptoms	0	0	0
Elevated LDH	4 (9%)	2(9%)	2 (9%)
Hb<10gr/dl	0	0	0
PLTs<100.000/μL	0	0	0
Neutrophils<1500/μL	0	0	0
Median lymphocyte count	4800/μL (1400-75600)	2750/μL (1400-4800)	8250/MI (5100-75600)
Paraproteinemia	20 (46%)	14(64%)	6(28%)
HCV infection	0	0	0
Autoimmune phenomena	3 (7%)	2(9%)	1(5%)
IPI			
Low	10 (23%)	2(9%)	8(38%)
Low – Intermediate	32(75%)	19(86%)	13(62%)
High-Intermediate	1(2%)	1(5%)	
%BM infiltration (median)	20 (5-80)	15 (5-60)	30 (10-80)

0216

VALIDATION OF THE INTERNATIONAL PROGNOSTIC SCORE IN PATIENTS WITH ADVANCED HODGKIN LYMPHOMA TREATED WITH ANTHRACYCLINE-BASED CHEMOTHERAPY

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Background. The International Prognostic Index (IPS), based on the analysis of 5023 patients treated before 1992, remains the most widely accepted prognostic system for advanced Hodgkin lymphoma. Nevertheless, IPS needs to be verified in independent patient series homogeneously treated with anthracycline-based chemotherapy (mostly ABVD) with or without radiotherapy. Few validation studies have been published so far with conflicting results**. Aims.** To assess the applicability of IPS in a relatively large series of patients with advanced HL under treatment with ABVD or equivalent regimens. **Methods.** In this retrospective study, we analyzed an homogeneous series of 510 patients with advanced Hodgkin lymphoma (stage IIB / III / IV) who were diagnosed and treated with anthracycline-containing therapy in 3 participating hospitals. Complete data for all parameters of IPS were available in 416 patients (82%), while at least half of the remaining patients were suitable for pooled analysis of the IPS (<3 versus ≥ 3 or <2 versus ≥ 2). Endpoints of the study were freedom from progression (FFP) and overall survival (OS). **Results.** Among 510 patients, 149 (29%) had stage IIB, 212 (42%) stage III, and 149 (29%) stage IV. The frequency of adverse IPS factors were: age ≥ 45 years 30%, male gender 57%, stage IV 30%, Hb <10.5g/dl 26%, leukocytosis ≥15x10^9/L 24%, severe lymphocytopenia 16% and albumin <4g/dl 65%. Only 23/416 patients (6%) had ≥5 risk factors. At a median follow up time of 81 months (6-308), 153 patients had refractory disease or relapsed and 107 had died. The 5-year FFP was 70% and the 10-year OS 73%. Among individual IPS factors, only stage IV was significantly correlated with FFP (p=0.0001), while the significance of hypoalbuminemia was marginal (0.05<0.0001 and p=0.02, respectively). Overall, the IPS was a statistically significant predictor of OS (p=0.01) but not FFP (p=0.11), as shown in the table. Instead, the discriminating ability of IPS was significantly improved for both OS (p<0.0001) and FFP (p<0.0001), when 306 patients with stage IIA were incorporated in the analysis. **Summary and Conclusions.** In an adequately sized series of patients with truly advanced stage HL, the IPS predicted OS and -less satisfactorily- FFP, without identifying subgroups of patients with very good or very unfavorable prognosis. Other conventional, biological or functional imaging-related factors need to be co-evaluated in order to identify sizeable subgroups of patients with sufficiently poor or favorable outcomes, suitable for treatment intensification or de-escalation respectively.

Table 1.

Comparison of FFP and OS in relation to IPS between the present study and the original publication (Hasenclever & Diehl, NEJM, 1998)

IPS	Patients [# (%)]	Patients(%) original publication	5/10 year FFP present study	5-year FFP original publication	10-year OS present study	5-year OS original publication
0	19 (5)	7	76 / 76	84	100	89
1	90(22)	22	78 / 78	77	92	90
2	99(24)	29	71 / 66	67	83	81
3	111(27)	23	65 / 64	60	76	78
4	73 (18)	12	63 / 54	51	81	61
5-7	23 (6)	7	76 / 76	42	90	56
p			0.11		0.01	

0217

HEALTH OUTCOMES IN HODGKIN'S LYMPHOMA SURVIVORS: INCIDENCE OF LATE COMPLICATIONS WITH A FOCUS ON CARDIOVASCULAR DISEASE AND IMPACT OF SCREENING AND INTERVENTION

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Background. More patients die from late effects of curative combined therapy modalities than disease progression in Hodgkin lymphoma (HL). Identification of risk factors and methods of risk reduction are of paramount importance to further improve long-term health outcomes. **Aims.** The primary aim of this study was to define the incidence and impact of late complications especially cardiovascular disease in patients cured of HL treated in a metropolitan health region over a period of 31 years.3. **Methods.** Patients were identified using the South Australian Cancer Registry and individual Hospital Cancer Registry. Patients older than 18 years and diagnosed after 1975 and in CR for 5 years were eligible to participate. The study was approved by the local Human Research Ethical Committee and informed consent obtained. Late complications and risk factors were assessed using modified Children's Oncology Group guidelines.4. **Results.** This program identified 158 patients with HL of which only 72 were fully evaluable at cut-off date (01.12.2011). The median age at diagnosis was 35 and at inclusion 52 years. Of all evaluable patients, 50 were alive at cut-off. 41 were not aware of late complications, and life-style and other factors modifying the risk. A long-term health plan existed for 9 patients only still seen by their hematologist/oncologist. The most frequently detected risk factors not including treatment were hypercholesterolemia (32), obesity (20), smoking (16) and hypertension (12). Late effects were common and led to significant morbidity and mortality. Second cancers occurred in 24, causing death in 17 patients. Screening revealed early cancer of breast, thyroid and bowel, respectively in 3 patients and one had relapse of HL. All underwent potentially curative surgical and medical treatment. Overt cardiovascular and cerebrovascular disease was detected in 15 and 6 patients, respectively of which 2 had not been diagnosed before. 2 patients died instantly from cardiac arrest due to myocardial infarction. 4 patients had asymptomatic valve disease and 3 atherosclerotic plaques now being reviewed for progressive disease annually. Hypertension increased the risk of cardiovascular disease, p=0.05. Radiotherapy, smoking, hypertension and hypercholesterolemia increased the risk of death from any cause, p<0.009.The SIR for secondary cancer was significantly raised as compared to the normal age-matched South Australian population (13.2). The same was true for cardio- and cerebrovascular disease (4.0 and 16.6, respectively). Radiotherapy appeared to be the single most important risk factor for cardiovascular disease in a chi square analysis with a p=0.015 using the Fisher Exact Test.5. **Summary and Conclusions.** Late effects are frequent and lead to excess and premature morbidity and mortality. A surprisingly high number of survivors are unaware of this risk. Reduction or avoidance of radiotherapy and management of risk through life-style choices such as smoking and other personal risk factors e.g. obesity, hypertension are of utmost importance as shown. Better means of follow-up, patient educationand retention, and integration of general practitioners in the treatment plan are a prerequisitefor successsful programs and need to be supported. Reduction of radiotherapy is being taken into account by current treatment concepts.

0218

PROGNOSTIC FACTORS IN PATIENTS WITH HODGKIN LYMPHOMA (HL) AND A NEGATIVE PET/CT AFTER ABVD CHEMOTHERAPY: POTENTIAL APPLICATIONS FOR THE DESIGN OF FOLLOW-UP STRATEGIES

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Background. Patients with HL who become PET/CT(-) after ABVD chemother-

apy are expected to have a very favorable prognosis. Nevertheless, it is known that relapse risk persists for prolonged time periods. Prognostic factors determining the outcome of PET/CT(-) patients have not been adequately studied, although they may be very important for patient monitoring and counseling. **Aims.** The aim of this study was to identify relevant prognostic factors in this specific subgroup of HL patients. **Methods.** 341 patients with HL were treated with 4-8 ABVD cycles from December 2004 to mid 2011. 268 responders (CR, CRu, PR) underwent PET/CT after ABVD, while 40 did not due to technical reasons. There were missing data for 8 patients, 4 died prematurely and 21 rapidly developed progressive disease before PET/CT. Among the 268 responders who were evaluated by PET/CT, 212 patients became PET/CT(-) after ABVD, forming the patient population of the present study. **Results.** The median age of 212 PET/CT(-) patients was 29.5 years (15-78), 51% were males, 98% had classical HL, 26% had stage III/IV, 30% had B-symptoms, 21% had ≥5 involved sites, 37% had anemia, 15% significant leukocytosis, 9% severe lymphocytopenia, 48% had ESR ≥50 mm/h, 54% albumin <4 g/dL, 20% elevated LDH and 25% high IPS (≥3). Eleven patients relapsed and 1 died in 1st CR. The median follow-up (after PET/CT) for the remaining 201 patients was 34 months (1-80). 96% of stage I/II patients received additional radiotherapy (median 3000 cGy). In contrast, 88% of advanced stage patients were not irradiated. Relapse free survival (RFS) was 98%, 96%, 94% and 92% at 1, 2, 3 and 4-6 years after the negative PET/CT scan respectively. Among the above potential predictive factors, univariate analysis demonstrated that an inferior RFS was predicted by advanced stage (3-year RFS 98% vs. 83% for stages I/II vs. III/IV, p=0.0002) and involvement of ≥5 sites (97% vs. 84%, p=0.02). Especially for the 19 stage IV PET/CT(-) patients, the 3-year RFS was only 72% vs. 89% for the 35 stage III patients. In multivariate analysis only stage III/IV was an independent prognostic factor for RFS (hazard ratio 8.8, p=0.006). At least 2/3 relapses in stages I/II and 1/8 in stages III/IV were detectable by clinical examination only; 1/8 advanced stage patients relapsed in a previously bulky site, which could have been irradiated. **Summary and Conclusions.** Patients with HL achieving a negative PET/CT after ABVD, have an approximately 8% cumulative risk of relapse during the subsequent 5 years. A negative PET/CT after 4-6 cycles of ABVD predicts an excellent short/mid-term outcome in patients with stages I/II, who are typically irradiated. On the contrary, stage III/IV patients rendered PET/CT(-) after 6-8 cycles of ABVD, who are not usually irradiated, still remain in a considerable risk of relapse approaching 20% within the initial 3 years. Therefore, although stage III/IV PET/CT(-) patients may require regular monitoring with imaging studies, this can probably be avoided in stage I/II PET/CT(-) patients who have received radiotherapy, in order to minimize further exposure to radiation and save health care costs.

0219

MYELOID IMPAIRMENT HAS COMPARABLE PREDICTIVE VALUE OF INTERIM-PET IN HODGKIN`S LYMPHOMA

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Background. ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine is considered the gold standard for Hodgkin`s lymphoma (HL), but a small fraction of patients fails to achieve long term disease control for either resistant or relapsing disease. BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) has been proposed for a better disease control, despite immediate and long term side effects. Thus, new biomarkers to identify risk-adapted strategies are an emerging need, including interim-PET after two courses of chemotherapy (PET-2). The ratio between absolute lymphocyte and monocytes count ALC/AMC in peripheral blood at diagnosis has been recently suggested. We have already demonstrated that a circulating sub-population of immunosuppressive myeloid cells (MDSC) has the ability to suppress T-cell immune response, and are increased in the peripheral blood of HL patients,In this retrospective study we evaluated sensibility and specificity of interim-PET, ALC/AMC and MDSC-count in peripheral blood at diagnosis to predict progression free survival (PFS). **Methods.** From September 2008 to October 2011, 52 consecutive patients affected by classical HL were evaluated. All patients were treated with standard ABVD therapy followed by consolidation radiotherapy in case of bulky presentation or residual tumor mass. Both baseline and PET-2 were performed using standard technique. In peripheral blood, we evaluated ALC/AMC ratio and circulating levels of immature MDSC by flow cytometry defined as $CD45^+$, $CD34^+$, $CD11b^+$, $CD13^+$, $CD14^-$. **Results.** After a median follow-up of 19.2 months (range, 3.5-36.4 months), 44 patients (84.6%) were in continued complete remission (cCR), 8 failed treatment (15.4%) after a median of 11.2 months.PET-2 was available for all patients. 6/52 patients (11.5%) were PET-2 positive and all of them shifted to BEACOPP scheme. PET-2 had sensitivity for predicting 2-year PFS of 50% (95% CI%, 15.7 to 84.3%) and a specificity of 95.5% (95% CI, 84.5% to 99.4%).48/52 patients had high ALC/AMC-DX ratio>1.1, defined in accord to Porrata, 2012, with sensitivity for predicting 2-year PFS of 93.8% (95% CI, 69.8 to 99.8), but a low specificity of 9.1% (95% CI, 4.2% to 16.5%).HL patients at diagnosis showed higher levels of MDSC when compared to matched for sex and age healthy controls (3.02±0.25 vs 1.62 ± 0.16, p<0.0001), with return to normal values within the first 2 cycles of chemotherapy (1.81 ± 0.31, p=0.013). High MDSC count was associated with a lower PFS. MDSC count had sensitivity for predicting 2-year PFS of 75% (95% CI%, 34.9 to 96.8%) and a specificity of 86.4% (95% CI, 72.7% to 94.8%). **Conclusions.** A low ALC/AMC-DX ratio correlates with a good prognosis, but in our series we were not able to confirm the high specificity of this marker and its predicting value was certainly inferior to PET-2. Despite the therapy switching in case of PET-2 positivity, PET-2 still maintained high sensitivity and specificity for predicting 2-year PFS. MDSC count had high sensitivity and specificity for predicting 2-year PFS, comparable with PET-2, with the advantage of low cost and immediate availability at diagnosis.

0220

IS THERE A ROLE FOR POSITRON EMISSION TOMOGRAPHY (PET/CT) IN THE INITIAL STAGING OF HODGKIN LYMPHOMA(HL) ?

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Background. PET/CT is crucial in the post-treatment evaluation of patients with HL, while several studies support the implication of interim PET/CT in the design of treatment strategy. Although baseline PET/CT is recommended, it is not considered mandatory for the initial staging of HL. Furthermore, the effect of baseline PET/CT on the choice of the first-line treatment has not been systematically studied so far. **Aims.** To compare the results of conventional clinical staging (CS) and PET-based staging (PET-S) in patients with previously untreated HL. **Methods.** Sixty-seven patients with HL, who were selected based solely on the availability of baseline PET/CT results, were evaluated by conventional and PET-based methods**. Results.** The median age of the 67 patients was 33 years; 40/67 were males. Based on CS, 8, 25, 17 and 17 patients had stage I, II, III and IV. The median number of involved anatomic sites was 1, 2 (1-5), 5 (2-14) and 7 (4-11) for patients with CS I, II, III and IV respectively. Based on PET/CT results, the corresponding figures were 2 (1-5), 3 (2-9), 7 (2-14) and 11 (5-14). The number of involved anatomic sites as determined by CS or PET-S were highly correlated (Spearman's rho 0.80). Compared to CS, PET-S resulted in stage change in 20/67 patients (30%): 17 were upstaged (25%) and 3 were downstaged (5%), as shown in the Table 1. Similarly, PET-S revealed more sites of involvement than CS in 50/67 patients (67%). Among 20 patients with potential stage shift, major treatment modifications could have been justified in 16 (24% of the total) based on PET-S. However, in only 7/16 a different therapeutic strategy was actually applied due to PET/CT findings. Among 33 patients with CS I/II, 21 (64%) actually received wider field irradiation, based on the identification of more involved sites by PET/CT. With respect to bone marrow (BM) findings, 43 patients had no evidence of bone/BM uptake and all of them had negative BM biopsies. A diffuse BM uptake was observed in 11 patients: 2/11 (18%) had histologically proven BM involvement. Multifocal bone/BM uptake was noted in 13 patients: 5/13 (38%) had histologically proven BM involvement. **Conclusions.** PET-S resulted in the identification of more disease sites in the majority of patients with HL. A stage shift was noted in 30% of patients, with the majority of them being upstaged. In everyday practice only 10% of the patients had their treatment strategy changed due to PET/CT findings, although less radical changes did occur in 2/3 of early stage patients due to the widening of radiotherapy fields. The variable use of PET/CT for the initial staging of HL and its heterogenous effect on treatment decisions

may render comparisons of retrospective series difficult. Furthermore, PET/CT-based design of radiotherapy may lead to the use of larger fields, in an era that minimization of radiotherapy is warranted. The optimal use of baseline PET/CT in HL needs to be further evaluated.

Table 1. Stage distribution according to conventional methods or PET/CT in 67 patients with HL.

		PET/CT-based stage				
		I	II	III	IV	Total
Clinical Stage	I	3	2	2	1	8
	II	0	16	1	8	25
	III	0	3	10	4	17
	IV	0	0	0	17	17
Total		3	21	13	30	67

0221

BEACOPP-14 VS BEACOPP-ESC IN PATIENTS WITH HODGKIN'S DISEASE FROM POOR-PROGNOSIS GROUP: INTERIM ANALISYS OF PROSPECTIVE RANDOMIZED MULTICENTER STUDY

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Background. treatment results in patients with high risk Hodgkin's lymphoma (HL) still are insufficient. The treatment intensification could be promised approach in this group of patients. **Aims.** To compare the treatment efficacy and toxicity with BEACOPP-14 and BEACOPP-esc regimens in patients with HL from high risk group. **Methods.** Since September 2008 until now 151 patients in 6 Ukrainian centers from 18 to 65 years old (median 29 year), 58 male and 93 female with stage IIB with≥1 unfavorable factor and stage III-IV were included to the study. Patients were randomized to receive BEACOPP-14 (67 patients, totally received 424 cycles, 6,32±1,09 cycles per patient) or BEACOPP-esc (84 patients, totally received 459 cycles, 5,46±0,92 cycles per patient). The treatment efficacy in both groups was evaluated according to Cheson criterias (1999, 2007). Toxicity rate was evaluated with NCI-CTC criteria V.3.0. After completion of chemotherapy patients with initial sites >5 cm, residual lymph nodes >2 cm and PET-positive sites received radiotherapy (30-36 Gy). Additionally the similar group of patients, who received the therapy with ABVD, was selected for the historical control from National Cancer-Register.

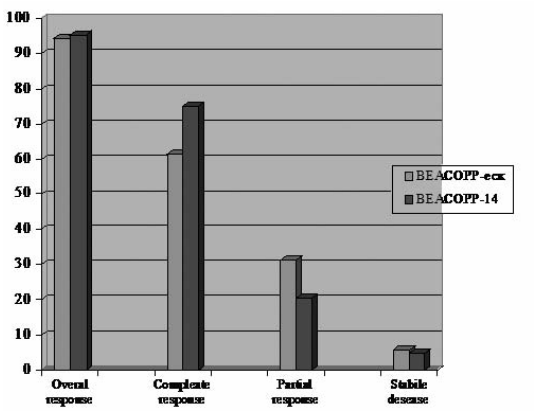

Figure 1.

Results. The treatment of patients from high risk group with both regimens was high-efficient. The response rate after the completion of chemotherapy is offer on the diagram. The ORR after the end of the treatment was 95,32 % in the group of BEACOPP-14 and 94,29 % in the group of BEACOPP-esc, CR rate was 75 % and 61,4 %, respectively (p>0,05). Observation period was 4-41 months (median 24 months). 2-year OS was 95.55% in the group of BEACOPP-esc and 100 % in the group of BEACOPP-14 (p> 0.05). In the group of historical control ORR was 80.39% (p<0.05), 2-year OS was 91,8% (p> 0.05). 2-year PFS was 94.91% in the group BEACOPP-esc, 97.95% in the group of BEACOPP-14 (p> 0.05) and was significantly lower in the group of ABVD (84,6 %, p<0.05). Toxicity of the therapy with BEACOPP-esc was higher then BEACOPP-14 due to hematological toxicity. The rate of anemia was higher in the group of BEACOPP-esc (63.18% vs. 54.25%, p<0,05), but the anemia of grade 3-4 was comparable 18,08 vs. 19,45%, respectively (p>0.05). The rate of neutropenia was also higher in the group of BEACOPP-esc (72.33% vs. 62.09%, p<0,05), but the neutropenia of grade 3-4 was comparable 46,3 vs. 42,48 % (p> 0.05). Thrombocytopenia rate was comparable in both groups (27.65% vs. 19.9%, p>0.05). In the treatment with BEACOPP-esc the rate of infectious complications was higher (13.09% vs. 4.47%, p<0,05)**. Conclusions.** ORR, CR and PFS were comparable in both groups of BEACOPP-esc and BEACOPP-14 and was significantly lower in the group of ABVD treatment. OS was comparable in all groups of patients. Toxicity rate was significantly higher in the group of BEACOPP-esc. These results need to be better studied in larger series with a longer follow-up.

0222

EXTRANODAL DISEASE IN HODGKIN'S LYMPHOMA: A RETROSPECTIVE ANALYSIS OF CLINICAL FEATURES AND OUTCOME

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Background. Hodgkin's lymphoma (HL) is a highly curable disease and 5-year survival is improving, being currently 86%. Cure rates of more than 90% for early HL and more than 70% for those with advanced HL are expected. At presentation, HL is usually supradiaphragmatic, with contiguous spread often occurring predictably from one nodal group to the next along the lymphatic pathways. HL is usually almost entirely confined to the lymph nodes. Extranodal involvement is much less common in HL than in non-Hodgkin lymphoma. Extranodal invasion of adjacent tissue is seen in up to 15% of cases. **Aims and Methods.** To further assess the presenting features and the prognostic significance of extranodal disease in HL we performed a retrospective single institution study of 524 cases with a median follow-up of 12 years.The median ages were 40 years (range, 14-83 years) for men and 34 years (range, 16-77 years) for women; 393 (75%) were younger than 45 years of age. Stages III-IV were present in 340 pts (65%), bulky disease in 204 pts (38%), extranodal disease in 61 (12%), 303 pts (58%) had an IPS score 0-2 (low-risk) and 214 (41%) had a score >2 (intermediate-high-risk). Combined radio-chemotherapy was administered in 340 pts (65%) and chemotherapy alone in 184 (35).

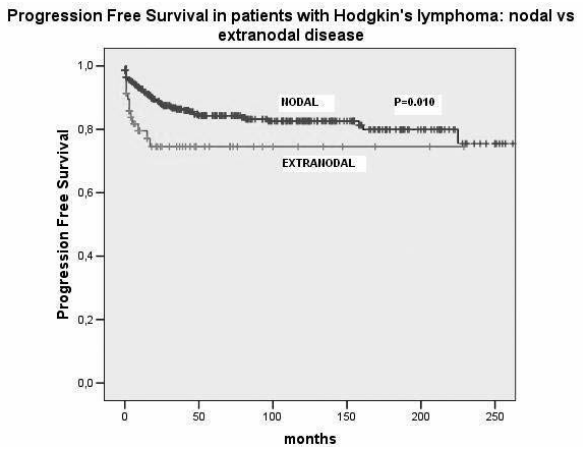

Figure 1.

Results. Extranodal disease was documented in 61 pts (12%) with a median age of 38 yrs (14-83 yrs), 39 (63%) had B symptoms, 27 (44%) had bulky disease, 29 (48%) had stage IV. Extranodal sites included the lung in 28 (41%), bone in 17 (25%); liver in 15 (21%), kidneys in 4 (6%), pleura in 4 (6%), pancreas in 1 (1%). The 61 patients with extranodal disease had poor prognosis compared with the nodal group (5 year progression-free survival [PFS], 73% versus 82%; p=0.010). Compared with the nodal subset, the extranodal patients presented more frequently with advanced stage disease (79% vs 21% p<0.001), a significantly higher (>2) HD-score (35.5% vs 10.3% p<0.001), B symptoms (63% vs 46% p=0.020), had a higher serum lactate dehydrogenase level (38% vs 18% p=0.014), a higher βeta2microglobulin level (33% vs 15% p=0.003), and a higher ESV level (64% vs 37% p=0.001). Complete remission (CR) rates in the extranodal and the nodal subsets of patient were 56% vs 71% (p=0.003), respectively. **Conclusions.** In our study extranodal disease in pts with HL is a rare occurrence (12%) associated with a poor clinical outcome. Further multicentric studies are needed to confirm our data.

0223

PRELIMINARY RESULTS OF NEW CHEMOTHERAPY REGIME 6 EA(50)COPP-14±RT IN PATIENTS WITH ADVANCED STAGE HODGKIN LYMPHOMA

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Introduction: Intensified chemotherapy with eight cycles of $BEACOPP_{escalated}$ or BEACOPP-14 in advanced stage Hodgkin Lymphoma (HL) is highly effective but also associated with relevant treatment related toxicity. High level pulmonitis in patients after bleomycin-consider cycles, especially with additional radiotherapy for mediastinal lymph nodes decrease quality of life. The clinical trial realized of the GHSG presented that exception of bleomycin from ABVD don't decrease efficiency of treatment. We excluded bleomycin from BEACOPP-14, the number of cycles reduced tosix, but dose of doxorubicin increased to 50mg/m^2 - EA(50)COPP-14. The program includes 6 EA(50)COPP-14± RT for initial bulky and residual mass. **Methods.** Between July 2008 and May 2011, 98 patients with newly diagnosed, histology-proven HL, aged 17-48 years, in Ann-Arbor stage II with mediastinal bulky or extranodal lesions, or those in stage III or IV were included. Sixty four patients finished their complete treatment in January 2011 they follow up 4 months after the end of treatment. The median follow-up consisted 19 months (from 9 to 31 months). Toxicities estimated in 77 patients, who finished chemotherapy in May 2011. Five patients were excluded from evaluable due to different reasons. **Results.** Complete (CR) and uncertain complete remissions (uCR) achieve in 60 (94%) of patients, partial response - in 3 (5%), progressive disease - in 1 (1%). One patient has early relapse after PET-negative CR. Secondary neoplasm was detected in 1 patient. In May 2011 all patients are alive.Hematological toxicities estimated in 77 patients. Particularly, neutropenia of III-IV grades was determined in 58 (75%) of patients, thrombocytopenia III-IV - in 12 (15%), anemia III-IV - in 52 (67%). The most frequency of hematological toxicities occurred after 4-5 cycles. Severe infection complications appeared in 22% of patients. **Conclusions.** The first preliminary results of treatment EA(50)COPP-14±RT showed high immediate efficiency and moderate toxicity.

0224

CIRCULATING CELL-FREE DNA IS A GOOD PROGNOSTIC BIOMARKER FOR HODGKIN LYMPHOMA

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Background. Circulating cell-free DNA (cfDNA) derived from both neoplastic cells and inflammatory microenvironment has been shown to be significantly higher in cancer patients compared to healthy individuals and to decrease progressively during cancer remission. We have previously demonstrated that levels of plasma DNA were significantly higher in patients with lymphomas compared to controls and described associations to patient characteristics and prognosis (Hohaus et al, Ann Oncol 2009; 20:1408). **Aims**. In this study, we extended our previous observation on cfDNA levels in a limited cohort of HL patients to a larger prospectively evaluated group of HL patients, and correlated cfDNA levels in addition to clinical characteristics and prognosis to other biological parameters, as cytokine levels and tumor-associated macrophages. Methods cf DNA levels have been studied in pre-treatment plasma samples of 136 consecutive HL patients (62 males and 74 females) recruited at our Institution between 2004 and 2011, and 63 healthy volunteers (28 males and 35 females). Quantitative analysis of cfDNA has been assessed by a Sybr-Green based- qPCR assay for the *β-globin* gene (GenBank accession number U01317) and results are expressed as ng/mL. The coefficients of variability intra- and inter-assay were both <0.01. **Results.** The median plasma levels of cfDNA for HL patients were 23.56 ng/ml (range 1.05-656.04 ng/mL) and resulted significantly higher compared to controls (median 12.28 ng/mL, range 0.43-34.72 ng/ml; patients vs controls p<0.001). Plasma cfDNA levels at diagnosis were indicative of the tumor burden in our population, and were higher in patients with advanced disease (stage IIB-IV vs I-IIa, p=0.03), presence of B-symptoms (p<0.001), increased LDH levels (p<0.001), and an IPS score >2 (p=0.002). Moreover, levels of cfDNA correlated with plasma levels of the cytokines IL-6 (b=0.35, p<0.001), and IL-10 (available in 68 patients; p=0.02) and there was a trend for higher cfDNA levels in pts with a infiltration of tumor-associated macrophages (CD68+ cells) > 5% (available in 84 patients, p=0.06). Using the 95% upper level of controls as cut-point, patients with elevated cfDNA levels had an inferior event-free survival (EFS) (p=0.0001) and an inferior overall survival (OS) (p=0.009). In a multivariate analysis including advanced stage and adjusting the analysis for the type of chemotherapy regimen, elevated levels of cfDNA remained a prognostic factor for EFS (HR 8.2; C.I. 1.8-37.9; p=0.007) and OS (HR 6.3; 95% C.I. 1.3-33.3; p=0.03). **Conclusions.** The quantitative evaluation of plasma cell-free DNA provides a potential non-invasive biomarker for Hodgkin lymphoma, with a significant prognostic impact on patients' outcome.

0225

INTERDISCIPLINARY EVIDENCE-BASED GUIDELINE FOR DIAGNOSIS, THERAPY AND FOLLOW-UP OF ADULT HODGKIN LYMPHOMA PATIENTS

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Background. Hodgkin Lymphoma (HL) is one of the most common malignancies in young adults and has become curable for the majority of patients even in advanced stages. So far, there is no national or international evidence-based guideline giving recommendations for clinical practice in the treatment of HL patients. To improve and standardize diagnosis, therapy and follow-up for these patients, a clinical practice guideline was developed and consented by all major medical societies. This guideline was funded by the German Program for Guidelines in Oncology Implementation. **Aims.** To emphasize the clinically most relevant guideline recommendations related to diagnostics, treatment and follow-up of adult HL patients. **Methods.** Based on systematic literature searches in CENTRAL and MEDLINE, the currently available literature was classified, appraised for quality criteria and summarized in evidence tables. The intensive collaboration between the methodological experts in the Cochrane Haematological Malignancies Group, the clinical experts in the multi-disciplinary working-groups and the German Hodgkin Study Group ensured high quality as well as clinically relevant and up-to-date guideline drafts. The recommendations generated this way were graded for clinical and quality aspects and discussed in an interdisciplinary consensus-conference, and subsequently have been adapted and finalized. At the final consensus conference, a total of 180 evidence-based recommendations were issued. **Results.** There is a strong consensus that all patients should be offered the possibility to be treated within a clinical trial. Chemotherapy is the mainstay of treatment for all stages of HL. Two cycles of ABVD followed by involved-field radiotherapy (IF-RT) at 20Gy are strongly supported by the available evidence in early favorable stages. Early unfavorable stages should be treated with 2 cycles of BEACOPP escalated followed by 2 cycles of ABVD and IF-RT of 30Gy. The current recommendation for advanced stage patients is 6 cycles of BEACOPP escalated followed by 30Gy radiotherapy on PET-positive residual mass. There is unclear evidence for the additional value of PET, and the recommendations differ between staging, interim and follow-up evaluations. Specific guidelines are given for relapsed patients, those with nodular lymphocyte predominant HL, elderly patients and those with co-morbidities such as HIV. Health care professionals should encourage patients to exercise and should discuss the potential harms of complementary medicine with the patient. Moreover, fertility aspects have to be discussed with the patients before starting any treatment. Structured follow-up care should be provided and should, in particular, detect relapses, long-term organ toxicities and secondary malignancies. **Summary and Conclusions.** The first evidence-based guideline on the treatment of patients with HL translates scientific evidence and expert knowledge into precise recommendations for these patients into clinical practice. In addition to this clinical value, the guideline will enable healthcare professionals to improve patient information and quality management.

Aggresive lymphomas 1

0226

IMMUNOGLOBULIN HEAVY CHAIN/LIGHT CHAIN PAIRS MEASUREMENT IS ASSOCIATED WITH SURVIVAL IN DIFFUSE LARGE B-CELL LYMPHOMA: ANALYSIS OF A LARGE COHORT INCLUDED IN THE LNH03-B PROGRAM OF THE GELA

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Background. Some biomarkers evaluable in the serum of diffuse large B-cell lymphoma (DLBCL) patients have been identified as prognosis factors and some of them can be routinely tested. These markers reflect the secretion or components of the tumor cells, nutritional status, tumor microenvironment, or can be related to secretion of pro-inflammatory cytokines. Elevated Serum free light chain (FLC) has been recently associated to an unfavorable prognosis in DLBCL. **Aims.** The aim of this study was to determine the clinical and prognostic relevance of a quantitative assessment of intact circulating immunoglobulin (Ig) using Ig heavy chain/light chain pairs (HLC) serum measurements in DLBCL patients. **Patients and Methods.** 407 adult patients (median age: 59y, 18-93) included in the LNH03-B clinical trial program with confirmed DLBCL were analyzed. The program initiated in 2003 included five clinical trials designed according to age and age-adjusted international prognostic index (aaIPI), providing a large cohort of patients with a long follow-up and being representative of the DLBCL population treated by rituximab (R) and anthracycline-based regimens. 244 were treated by R-CHOP/ R-mini-CHOP and 163 by ACVBP/R-ACVBP regimens. Serums were collected at the time of inclusion before any chemotherapy and used for FLC/HLC measurements performed by FREELITE™ and HEVYLITE™ assays. Published reference ranges were used to define an elevated FLC, an abnormal Kappa (K)/Lambda (L) FLC ratio and abnormal Ig(A-G-M) K/L ratios. **Results.** An abnormal K/L FLC ratio was observed in 38 patients (9.4%). 17 patients (5%) displayed an elevation of both K and L FLC and 76 (18.8%) an elevation of K or L FLC. Abnormal IgAK/L, IgMK/L and IgGK/L ratios were observed in 12 (2.9%), 78 (19%) and 26 (6.4%) patients, respectively. Patients with abnormal IgMK/L or abnormal FLC ratio displayed more frequently adverse characteristics including elevated LDH level (p =0.007), older age (p<0.001), elevated Beta2 microglobulin (p<0.001), or high IPI (p<0.001). No correlation was observed between lymphocyte count, creatinine level, albuminemia and FLC/HLC measurements. Patients with abnormal IgMK/L ratio had an inferior PFS in the overall cohort (5y-PFS CI95% 44.9% vs 69.4%, p = 0.0003) and OS (5y-0S CI95% 50.8% vs 78.3%, p=0.0003) and in the R-CHOP cohort (5y-0S CI95% 43.5% vs 70.6%, p=0.003) as compared with patients with normal ratio. Elevated FLC (5y-OS CI95% 36.6% vs 74.4%, p<0.001), abnormal K/L FLC and abnormal IgGK/L ratio were also associated to an unfavorable outcome. In a multivariate analysis including abnormal IgMK/L ratio, elevated FLC and aaIPI, an abnormal IgMK/L ratio (HR =1.6 CI95% 1.1-2.3, p=0.02) remains predictive of a shorter OS. Gene expression profile was performed in a subset of 58 patients using microarrays (Affymetrix U133+2). Patients with abnormal IgMK/L ratio tend to belong more frequently to the ABC subtype (p=0.1) and expressed at a higher level the Cμ gene (p=0.01), indicating that IgMK/L ratio measurement is partially driven by tumor biology. **Conclusions.** Both elevated serum FLC and abnormal IgMK/L ratio are associated with unfavorable outcome in DLBCL treated by immunochemotherapy and can be used as simple circulating biomarkers.

0227

DECREASED B AND T CELL REPERTOIRE DIVERSITIES ARE ASSOCIATED WITH INFECTIOUS RISK IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) PATIENTS

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Background. Despite the progress made in the management of patients with lymphoid malignancies, infections remain a frequent complication encountered during or after therapy. Beside chemotherapy induced neutropenia, immune dysfunctions may also contribute to the emergence of infections. Serum immunoglobulin levels are frequently altered and changes in T cell populations and functions are described in the context of several B cell neoplasms such as inverse circulating CD4/CD8 ratio, skewed distribution of regulatory T-cells (Treg) CD4+, all of them potentially contributing to infection vulnerability.Aims : In order to better characterize the adaptive immune cells and the infectious risk in patients with lymphoid malignancies, detailed molecular analyses of B- and T-cell receptor repertoires were analyzed on PBMC samples at diagnosis and correlated with the occurrence of infection. **Methods.** In a retrospective study, DNA extracted from PBMC obtained from 83 patients with lymphoid malignancies (CLL n=27; DLBCL n=21; follicular lymphoma n=37) at diagnosis (and 10 controls) was analyzed using a standardized investigation kit (ImmunTraCkeR). This device was designed to analyze the entire IGHVJ and TCRB VJ genes combinatorial diversity using multi-N-plex PCR amplification. The distribution of the IGH and TCRB combinatorial diversity was plotted together with CD19+ and CD3+ lymphocytes, respectively. Results were correlated with the occurrence of WHO grade 2 or higher infections during the first 6 months of the disease. **Results.** Both the IGH and TCRB repertoire were found to be profoundly altered at diagnosis. The median percentages of IGH combinatorial diversity were 20.83%, 46.88% and 39. 58 % in CLL, DLBCL and FL patients, respectively. The median percentage of TCRB combinatorial diversity were respectively 33.33%, 13.04% and 9.06%. All these values (except for IGH in DLBCL patients) were significantly different (Willcoxon test) from those observed in healthy individuals (54.17% and 59.60% for IGH and TCRB, respectively).Using ROC curve analysis, we attempted to determine optimal thresholds of IGH and TCRB diversity associated with the risk of infections. In DLBCL patients (but not in FL and CLL patients), IGH diversity below 51% and TCRB diversity below 10% were determined as optimal threshold for the occurrence of infections. When combining these 2 parameters (IGH < 51% and TCRB < 10%), a highly significant difference in the risk of developing an infection during the 6 first months of treatment was observed (Log-rank test, P=.026). Of note, lymphopenia at diagnosis was not associated with this risk in this cohort. **Conclusions.** These early results indicate that 1) both the B- and T-cell receptors repertoires are profoundly modified in patients with lymphoid malignancies at diagnosis 2) these modifications could be associated in DLBCL patients with the risk of developing an infection during treatment. A prospective series to further expand and confirm these findings is currently under way. Such confirmation may eventually help to consider anti-infectious prophylaxis or treatment choice in patients with lymphoid malignancies. These analyses may also prove to be useful to investigate if the immune cells dysfunctions may also be associated with the clinical outcome of patients.

0228

PROTEIN KINASE CK2 INHIBITORS CAUSE MANTLE CELL LYMPHOMA CELL APOPTOSIS AND SYNERGISTICALLY ENHANCE THE CYTOTOXIC ACTIVITY OF PROTEASOME INHIBITOR BORTEZOMIB

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Background. Bortezomib (BZ) is a proteasome inhibitor currently employed in the therapy of multiple myeloma and in relapsed/refractory mantle cell lymphoma (MCL). However, in MCL complete remission rates are low and the duration of response relatively short. BZ causes cell apoptosis through different mechanisms, which are only partially known. Mechanisms of BZ resistance could involve the upregulation of pro-survival signaling cascades. Protein kinase CK2 is a growth-promoting protein that has been implicated in human solid and hematologic tumors; phase I clinical trials are ongoing utilizing oral ATP-competitive CK2 inhibitors in multiple myeloma and other malignant neoplasias. Despite CK2 has been shown to regulate signalling pathways playing a relevant role in MCL patho-biology, it is currently unknown whether this druggable kinase could promote MCL survival and be involved in BZ-induced cell death.

Aims. In this study we have investigated CK2 expression, cellular localization and its role in MCL survival. We analyzed whether CK2 takes part in BZ-induced MCL cell apoptosis and we studied whether blocking CK2 could influence pro-survival signalling pathways, which could account for cell resistance to BZ.METHODSBZ-sensitive (Jeko-1 and Granta-519) and BZ-resistant (Rec-1) MCL cell lines and freshly isolated B lymphocytes from healthy donor and MCL patients were cultured as per standard methods and exposed to different doses of two CK2 inhibitors (K27 and CX4945) and BZ. Cell proliferation was measured by [^{3}H]-thymidine incorporation assay and the combination index was calculated to determine whether CK2 and BZ inhibition has a synergistic effect. Cell growth and viability was assessed upon the different treatments by annexin V and propidium iodide staining, evaluation of mitochondrial potential depolarization and FACS analysis, western blotting of PARP cleavage and apoptosis related protein expression. Survival signaling pathways were studied with western blot analysis. **Results.** CK2 was highly expressed in MCL cells and its inhibition caused apoptosis and cell proliferation arrest. Simultaneous inactivation of CK2 (using two different inhibitors) and the proteasome resulted in a synergic anti-lymphoma effect with a calculated combination index inferior than 1 both in BZ-sensitive (Jeko-1) and in BZ-resistant (Rec-1) cell lines. The rate of BZ-induced MCL cell apoptosis was significantly increased by the simultaneous inhibition of CK2 and the proteasome in all the MCL models tested. The synergistic effect between the proteasome and CK2 inhibition was not present in healthy donor derived C19 B lymphocytes. Mechanistically, CK2 inhibition produced a reduction of phospho Ser 536 and phospho Ser 529 p65 NF-κB subunit levels in MCL cells. Remarkably, the simultaneous treatment with BZ and CK2 inhibitors determined a further reduction of this phosphorylated proteins. **Summary and Conclusions.** These results indicate that protein kinase CK2 is essential for MCL survival, protects from BZ-induced apoptosis and modulates pivotal growth promoting signaling pathways in MCL cells, such as the NF-κB cascades. Our findings suggest that CK2 inhibition could represent a rational therapeutic option for MCL and offer the groundwork to design novel combination treatments for this disease.

0229

IMPROVED OUTCOME OF ELDERLY DLBCL PATIENTS WITH 6XCHOP-14 AND 8 RITUXIMAB (R) APPLICATIONS GIVEN OVER AN EXTENDED PERIOD (SMARTE-R-CHOP-14 DSHNHL TRIAL) IS DUE TO BETTER RESULTS OF MALE PATIENTS

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Background. 6xCHOP-14 with 8xR given over an extended period improved 3-year EFS (67% vs. 54%; p=0.030) and OS (80% vs. 67%, p=0.034) of poor-prognosis patients (IPI=3-5) in the SMARTE-R trial compared to the RICOVER-60 trial where patients received 8xR every 2 weeks. **Aims.** Because we had recently shown (Mueller et al., Blood 2012) that elderly male patients have a faster R clearance (12.68 ml/h vs. 8.21 ml/h; p=0.003) and shorter serum elimination half life ($t_{1/2ß}$=24.7 vs. $t_{1/2ß}$=30.7 days; p=0.003) than females, we analyzed whether these differences translated into different outcomes by comparing the results achieved by elderly female and male patients in the RICOVER-60 and SMARTE-R trials. **Methods.** In SMARTE-R, 189 evaluable elderly (61-80 y) pts. with DLBCL received 6 cycles of 2-weekly CHOP-14 combined with 8xR on days -4, -1, 10, 29, 57, 99, 155, and 239. The primary endpoint was event-free survival (EFS). 306 pts treated within the RICOVER-60 trial with 6xCHOP-14 + 8 R given on days 1, 15, 29, 43, 57, 71, 85 and 99 served as controls. **Results.** The 3-year EFS of 51 poor-prognosis male patients in SMARTE-R was 67% compared to 47% of 66 poor-prognosis male patients treated in RICOVER-60 (p=0.037); the respective figures were 71% vs. 53% (p=0.051) for PFS and 80% vs. 60% (p=0.027) for OS. In contrast, female poor-risk patients had only a small benefit from the extended rituximab exposure in SMARTE-R (n=48) compared to RICOVER-60 (n=57): 67% vs. 61% (p=0.354) for EFS; 71% vs. 67% (p=0.489) for PFS; and 80% vs. 76% (p=0.528) for OS. **Conclusions.** Elderly male patients with poor-prognosis DLBCL who have a faster R clearance and shorter R serum elimination half life than female patients benefit significantly from the longer R exposure in SMARTE-R with a gain of 20% in 3-year OS, while the outcome of female patients was only slightly improved. Even though R maintenance has failed to demonstrate any benefit in the primary treatment of DLBCL to date, these results underline the importance of a minimum exposure time of R in order to exploit its full therapeutic potential in DLBCL. *Supported by Deutsche Krebshilfe.*

0230

THE CANADIAN TOSITUMOMAB AND I131 TOSITUMOMAB (TST/I131-TST) EXPERIENCE: FIVE YEAR SURVIVAL DATA

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Background. Indolent NHL is characterized by relapses and remissions, responding less to each successive line of therapy. Radiotherapy in NHL is generally limited to localized disease or palliation, toxicity increasing with field size. CD20 immunotherapy has revolutionized NHL specifically targeting B cells. Combining these modalities as radioimmunotherapy is highly active but remains to be fully characterized. Mature survival data in this setting is very limited. The Canadian experience with 5-year follow-up can now provide some insights in this regard. **Aims.** Assess overall survival (OS) and progression free survival (PFS) at 5 years from treatment with TST/I^{131}-TST, explore predictors of OS and PFS, and update toxicity. **Methods.** The largest phase II open label study of TST/I^{131}-TST in indolent NHL was conducted at 12 Canadian centres. Patients with >2 prior lines of therapies, at least one including rituximab, responding to their last treatment were enrolled from April 2004 to February 2007. **Results.** 93 patients were included in the intent-to-treat population analysis. Median age was 59 years (32-78). ECOG status was0 in 55.9% and1 in 37.6%. Follicular NHL was present at original diagnosis in 94.6%, 78.5% with pathologic grade 1/2 histology. Bone marrow involvement was documented in 25.8%. The median duration of disease was 4.9 years (0.8-22.7). The median number of prior therapies was 5 (2-14) with 39.8% enrolled within 6 months of their last therapy. 25.8% had received prior radiotherapy.Study defined 6 months response rate was 43.0% (95% CI 32.8-53.7; 4.3% CR), 50% (38.6 - 61.4) in 80 evaluable patients. With 5 years of follow up, the median duration of response was not reached (95% CI 17.4-NR). Median PFS was 12.0 months (95% CI 7.7-17.5). Median OS was 59.8 months (95% CI 31.7-NR, 1.2-62.9+ months, Figure 1). In multivariate analyses hemoglobin (<120g/L), bulky disease (>5cm), and BSA (>2.2 m^2) were identified as significant predictors of OS, whereas LDH (>ULN), bulky disease (>10 cm), BSA (>2.2m^2), and number of prior therapies (>4) predicted PFS.There were 47 deaths, 40 of progressive disease and 7 of other causes (4 infectious, 1 aspiration, 1 leukemic transformation of MDS, 1 respiratory failure) with 5 of these 7 having prior progression. While on study (to 6 months), treatment was well tolerated. 13 patients (14%) had at least one SAE, six considered related to study medication by investigators. 33% had a grade 3/4 AE, including neutropenia (1%/5%), anemia (3%/0%) and thrombocytopenia (0%/1%). One febrile neutropenia was observed. After 6 months, there were four further SAEs reported in three patients, including MDS (2 cases), fatal sepsis (in one MDS patient), and left ventricular dysfunction. The MDS patients had 3 and 6 treatment lines prior to receiving TST/I^{131}-TST. Median TSH remained normal. Nine (9.7%) patients had two consecutive TSH measurements above UNL. **Conclusions.** Treatment with TST/I^{131}-TST was associated with durable responses as well as prolonged OS and PFS, with no unexpected toxicities in these heavily pretreated indolent NHL patients. Predictors of outcome included tumour bulk, BSA, hemoglobin, LDH and prior number of therapies.

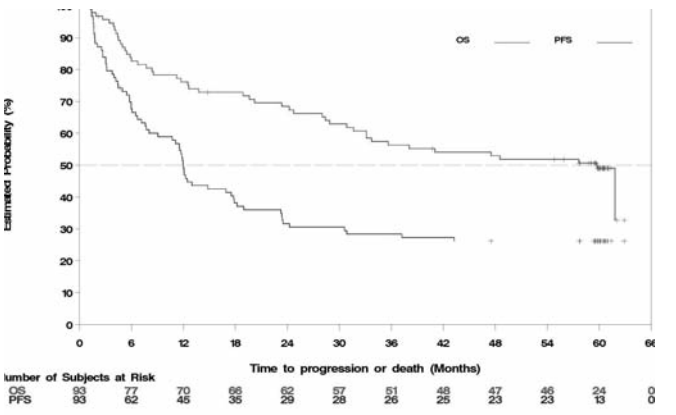

Figure 1.

0231

LONG TERM FOLLOW-UP OF DOSE-DENSE CHEMOIMMUNOTHERAPY FOLLOWED BY HIGH-DOSE CHEMOTHERAPY PLUS AUTOLOGOUS STEM CELL TRANSPLANTATION IN UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA WITH POOR PROGNOSIS

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Background. Diffuse Large B-cell Lymphoma (DLBCL) patients with poor prognosis (age-adjusted International Prognostic Index, aa-IPI 2-3), had a dismal prognosis if treated with conventional chemotherapy. Some phase II studies and preliminary data of randomized phase III study conducted in Rituximab-era suggested that the addition of rituximab to high-dose chemotherapy was effective in DLBCL with poor prognosis at diagnosis, but results are controversial. **Aims.** of this study was to perform a long term follow-up of a series of patients included in a multicenter phase II Italian study (Vitolo U et al, Haematologica 2009) and to analyze the risk of central nervous system (CNS) relapses and the incidence of late toxicities. **Methods.** Inclusion criteria were: young patients (age, 18-60) with stage III-IV diffuse large B-cell lymphoma at aa-IPI 2-3. Treatment plan was: four courses of bi-weekly Rituximab-intensified CHOP (R-iCHOP14) followed by two courses of rituximab plus mitoxantrone, high-dose cytarabine and dexamethasone (R-MAD) and high dose chemotherapy BEAM plus autologous stem cell transplantation (ASCT). Updated data regarding of survival, CNS relapses and late toxicities were recorded on November 2011. **Results.** From 2002 to 2005, 112 patients were treated according to the phase II trial. Clinical characteristics were: median age 47 (36-52) years; aa-IPI 3 44%; bone marrow involvement 21%. With a median follow-up of five years, 5-year Overall Survival was 79% (95% CI: 70% - 86%). In a multivariate analysis, the risk of death was adversely influenced by age with a progressive increase of five years at diagnosis (p.008) or aa-IPI3 (p.001). Three patients experienced CNS relapses. Only one of the three patients received CNS prophylaxis with intrathecal Methotrexate, even if all of them were at risk for CNS relapse according to Italian Society of Hematology guidelines. Cumulative incidence of CNS recurrence at 10 years for R-iCHOP14+R-MAD+BEAM and ASCT was 3.6% (0 to 7.8). Most frequent late toxicities were dyslipidemia and secondary amenorrhea. Regarding to secondary malignancies, myelodisplasia or acute myeloid leukemia were recorded in two patients, at a median time of seven years off therapy. The actuarial risk of secondary malignancies at 10 years was 4.2% (0 to 10). **Conclusions.** The addition of Rituximab to dose-dense iCHOP followed by R-MAD and high-dose chemotherapy plus BEAM and ASCT improved the outcome in young untreated DLBCL patients at poor prognosis, with an acceptable risk of secondary malignancies and late toxicities. A careful identification of patients at risk could avoid the risk of CNS relapse.

0232

CLINICAL IMPORTANCE OF METABOLIC TUMOR VOLUME BY PET/CT IN LOCALIZED EXTRANODAL NK/T CELL LYMPHOMA, NASAL TYPE

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Background. Front line use of radiotherapy produced superior survival compared to initial chemotherapy in localized extranodal NK/T cell lymphoma (ENKTCL) in previous studies. Poor drug delivery owing to tissue necrosis related to angiodestruction and frequent expression of multidrug resistance (P-glycoprotein-positive) phenotype might be the important contributing factors. The use inadequately accurate imaging for radiation therapy volume delineation might lead to missed tumor or irradiation of excess volumes of normal tissues. Recently, PET/CT would be a promising tool for the radiotherapy planning to active lesion for ENKTCL. Furthermore, it was very difficult to obtain local control of the bulky lymphoma in a recent study.Aim. The objective of the present study is to investigate whether metabolic tumor volume (MTV) would be a prognostic factor in limited stage ENKTCL **Methods.** Seventy-four patients with ENKTCL in nasal area underwent PET-CT at diagnosis were enrolled for the present study. Treatment modality were given as follows: chemotherapy (CT) followed by radiotherapy (RT) (34 patients), RT followed by CT (20 patients) and CT alone (20 patients). MTV was delineated on the PET/CT images by extranodal region of nasal area equal or greater than standard uptake volume (SUV) 2.5. The final MTV was calculated by using fusion software (Syntegra, version 2.1E, Philips Co.).Results. ROC curve analysis was used to calculate the accuracy of ideal cut-off value to distinguish between low MTV and high MTV group. Various cut-off values of MTV were used to obtain a reasonable balance of sensitivity and specificity. 31.2 cm^3 of various values acquired a sensitivity of 90.3% and specificity of 58.1%. The median age was 58.5 years (range, 23-74 years) and male : female ratio was 45 : 29. The patients of ECOG performance status above grade 2 were 9 patients. Patients including stage IE and IIE were 42 : 33. The clinical outcomes were compared according to several prognostic factors such as age, high performance status (PS) grade above 2, high stage such as IIE, high international prognostic index (IPI) above 2, high LDH level above upper normal limit, local tumor invasiveness (LTI), up-front radiotherapy (RT) and high MTV above 31.2cm^3. Univariate analysis revealed that other seven factors except age had significant prognostic values in PFS and OS (PFS, high PS [p=0.006], High stage [p=0.044], high IPI [p=0.010], high LDH [p=0.017], LTI [p=0.003], up-front RT [p=0.003] and high MTV [p<0.001]; OS, high PS [p=0.004], high stage [p=0.017], high IPI [p=0.010], high LDH [p=0.009], LTI [p=0.001], up-front RT [p=0.002] and high MTV [p<0.001]). In multivariate analysis, up-front RT (PFS, HR=0.232, 95%CI=0.068-0.798, p=0.020; OS, HR=0.214, 95% CI=0.050-0.926, p=0.039) and high MTV (PFS, HR=2.962, 95%CI=1.101-7.968, p=0.032; OS, HR=3.345, 95% CI=1.276-8.708, p=0.014) were significant prognostic factors in PFS and OS.Conclusion. The present study showed that up-front RT and tumor burden at diagnosis measured by PET/CT had a significant prognostic values in ENKTCL.

0233

RITUXIMAB MAINTENANCE THERAPY IN DIFFUSE LARGE B-CELL LYMPHOMA IN A MULTICENTER PROSPECTIVE RANDOMISED PHASE II STUDY

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Background: Clinical and pharmacokinetic data suggest that the effect of rituximab could be improved by prolonged exposure to the drug. To test for this hypothesis we performed a prospective randomized trial of rituximab maintenance therapy versus observation in patients (pts) with CD20+ B-cell Non-Hodgkin-Lymphoma. **Methods.** After completion of standard treatment, pts with $CD20^+$ B-cell lymphoma were randomized to either observation or maintenance therapy with rituximab (375 mg/m^2) administered every 3 months for 2 years. Both pts after first line therapy and pts after relapse treatment were included in the study. Pts with aggressive lymphoma were enrolled if they had achieved a complete response (CR) after initial treatment. Pts with aggressive lymphoma with residual tumor mass underwent positron emission tomography (PET) and qualified for randomization if this examination showed no signs of tumor activity. Pts with indolent lymphoma were eligible for the study if at least a partial response (PR) was achieved. Primary endpoint of the study was progression free survival (PFS), secondary endpoints were time to progression (TTP), overall survival (OS) and response to treatment. **Results.** 326 pts were included in the trial. Complete data sets of 294 pts were available for analysis on an intention-to-treat basis. We here report on the subset of 145 patients with diffuse large B cell lymphoma (DLBCL). 73 pts were randomized to the treatment group and 72 pts to the observation group. 77 (53%) pts were male and 68 (47%) female, with no significant gender differences between treatment and observation groups (p=0.74, Fisher's exact test). The median age was 58.6 years. 130 pts had received one previous therapy, 12 pts two, and two pts three previous lines of therapy. At study entry, 116 pts were in CR, 15 pts in unconfirmed CR and 14 pts in PR. Age, sex, number of previous therapies and remission status were well balanced between the treatment and the observation group (p≥0.48). After a median follow up of 30 months, PFS was excellent with 91% in the treatment group and 86% in the observation group (p=0.38). There was no difference in OS between the two groups. In a multivariate Cox model, OS

and PFS were not different between men and women. The estimated hazard ratio of female vs. male was HR(F:M)=0.90 (p=0.90) for OS and HR(F:M)=0.49 (p=0.20) for PFS. However, interestingly there was a significant interaction (p=0.02) between therapy group and gender in regards to PFS with significant more events in men than in women in the observation group (HR(F:M) = 0.12; 95% confidence interval (CI) =(0.02, 1.00)), and no differences between men and women in the treatment group (HR(F:M) = 2.71 ; 95%CI=(0.50,14.9)). **Conclusions.** In this study, rituximab maintenance therapy did not improve PFS or OS in patients with DLBCL in general. However, in male patients a significant better PFS was found in the treatment group compared to the observation group (HR=0.21; p=0.03), suggesting a benefit of rituximab maintenance therapy for male patients with DLBCL.

0234

A PHASE II TRIAL ADDRESSING EFFICACY OF HELICOBACTER PYLORI-ERADICATING THERAPY AS UP-FRONT TREATMENT IN EARLY-STAGE GASTRIC DIFFUSE LARGE B-CELL LYMPHOMA: IMPLICATIONS OF ONTOGENETIC CLASSIFICATION

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Background. *Helicobacter pylori* (*Hp*) infection is associated with both marginal zone lymphoma of MALT-type and diffuse large B-cell lymphoma (DLBCL) of the stomach. While *Hp*-eradicating antibiotic therapy is the standard treatment for limited-stage MALT lymphoma, the role of this strategy in gastric DLBCL is still controversial and resulting successful from a few case reports and small monoinstitutional retrospective series. **Aims.** This is the first multicentre phase II trial addressing safety and efficacy of *Hp*-eradicating therapy as exclusive treatment for limited-stage gastric DLBCL in Western countries. **Methods.** Inclusion criteria were histopathologic diagnosis of DLBCL with or without concomitant MALT-type areas; gastric *Hp* infection; stage-I disease with or without concomitant perigastric lymph nodes diameter <1.5 cm; hemoglobin ≥9 g/dl; normal LDH serum level. Registered patients received clarithromycin 500 mg bid, tinidazole 500 mg bid and omeprazole 20 mg bid, for 7 days. Objective response and bacterial eradication were assessed by gastric endoscopy-ultrasonography, biopsies and breath test after one and two months from antibiotics. Patients who achieved complete remission (CR) were referred to observation, patients in partial response (PR) received four doses of rituximab and patients with stable (SD) or progressive (PD) disease received anthracycline-based chemotherapy ± radiotherapy. Immunohistochemical ontogenic classification of DLBCL (i.e. Germinal center-GCB) versus non-germinal center (i.e., non-GCB) followed criteria proposed by Hans et al. **Results.** Sixteen patients (median age 70; range 38-87; 11 males) were registered. Five patients presented concomitant MALT areas; ten patients had perigastric lymphadenopathies. *Hp* eradication was achieved in all cases. Histopathological material for Hans' classificationwas available in 14 cases. CD10, bcl-6, bcl-2, and MUM1 immunoreactivity was detected in 4 (29%), 10 (71%), 12 (86%), and 7 (50%) cases, respectively. Accordingly,7 patients displayed a GCB phenotype and 7 showed non-GCB phenotype. Median Ki-67 (MIB1) proliferation index was 70% (range 30-99%).Lymphoma regression was complete in 8 (50%) patients, and partial in 3 (ORR= 69%; 95%CI= 47%-91%), one patient had SD and 4 PD. Two of the three PRs achieved CR after rituximab (CRR= 63%; 95%CI= 39%-87%). At a median follow-up of 66 months (range 13-104), nine of the 10 CRs were relapse-free, with a median DFS of 74+ months. The non-responder patients and the relapsed one received R-CHOP-like chemoimmunotherapy ± radiotherapy, and remained disease-free at 13-90 months (median 46+). Response and relapse rates were not associated with the presence of concomitant MALT areas, perigastric lymphadenopathies or Hans' classification results. Fourteen patients are alive, with a 5-yr OS of 94%; no patient died of lymphoma. Two patients (77 and 78 years old) died of cardiac failure after salvage treatment and gallbladder cancer at 13 and 90 months, respectively. **Conclusions.** Patients with limited-stage, *Hp*-associated DLBCL ± MALT of the stomach can be safely managed with antibiotics alone. Responses were recorded both among GCB and non-GCB lymphomas as well. Half of treated patients achieved long-term remission without chemotherapy, a critical issue considering that two-thirds of patients are old. Unresponsive patients can be safely salvaged with conventional chemo-radiotherapy with unaltered chances of cure.

0235

IMMUNO-CHEMOTHERAPY CONTAINING RITUXIMAB IS ASSOCIATED WITH IMPROVED SURVIVAL IN PRIMARY CNS B CELL LYMPHOMA

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Background. Primary central nervous system lymphoma (PCNSL) is a rare and biologically distinct subset of non-Hodgkin lymphoma. Due to its low incidence, optimal treatment strategies remain uncertain and are currently based upon outcomes from retrospective series and small phase-II studies. In addition to improving survival outcomes in systemic B cell lymphomas, rituximab penetrates the CSF after intravenous administration and also reduces CNS relapse in high-risk systemic DLBCL. However its role in PCNSL remains uncertain. We report the results of a retrospective study examining the impact of rituximab, methotrexate, cytarabine and radiotherapy in patients managed with curative intent at four university teaching hospitals between 1996-2011. **Methods.** A retrospective study of consecutive cases of PCNSL treated at the participating institutions was performed in accordance with institutional ethical guidelines. Patients were included if they were HIV negative and had a first presentation of biopsy-proven primary CNS lymphoma or intravascular large B cell lymphoma according to WHO 2008 criteria. Patients were excluded if they were HIV positive or not treated with curative intent. Survival correlates were assessed by Cox regression using SPSS.

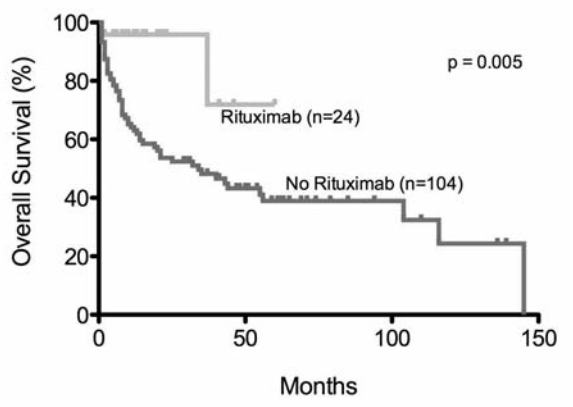

Figure 1. Overall survival of patients that were treated with curative intent and received rituximab.

Results. Of 164 patients, 30 were treated with palliative intent; 134 patients met the criteria for the study. The median age was 65 years (range 21-81), 49% male. At diagnosis, 31% had an ECOG performance status (PS) >1 and 43% an elevated serum lactate dehydrogenase (LD). Deep lesions, multiple lesions and cytology positive CSF involvement were found in 63%, 52% and 14% respectively. There was no difference in base-line characteristics between rituximab recipients and non-recipients. Histological diagnosis was DLBCL (122), T cell lymphoma (4), low-grade B cell lymphoma (1), indeterminate (3) and intravascular large B cell lymphoma (4).Chemotherapy regimens included single agent high-dose methotrexate (HD-MTX, ≥2g/m^2; n=83), combination chemotherapy including HD-MTX and cytarabine (37), other combination chemotherapy including HD-MTX (5) and combination chemotherapy without HD-MTX (8). Other front-line therapies included intrathecal MTX (43), radiotherapy (32) and rituximab (24). The median follow-up of surviving patients was 22 months. The 5 year overall survival (OS) was 44%.Univariate analysis revealed age ≤60 years (p=0.029), ECOG PS 0-1 (p=0.012), normal LD (p=0.027), diagnosis after 2004 (p=0.020), and treatment with high-dose MTX (p=0.045), cytarabine (p=0.013) and rituximab (p=0.005) as predictive of favourable OS. Cox regression analysis identified only rituximab (HR 5.3, 95% CI 1.3-21.9; p=0.002) and ECOG PS (HR 1.9, 95% CI 1.1-3.1; p=0.026) to be independent predictors of OS. At census, 22 of 24 patients treated with rituximab-containing regimens were still alive with a median follow up of 15 months. No differ-

ence in survival was observed between patients receiving HD-MTX 8 g/m^2 versus 2-3.5g/m^2 (p=0.81). Radiotherapy and intrathecal MTX were not associated with improved survival (p=0.444 and p=0.529). **Conclusions.** This retrospective analysis suggests that the addition of rituximab to HD-MTX based chemotherapy in patients with aggressive B-cell PCNSL was associated with an improved OS. Further studies are underway to validate these findings prospectively.

0236

NON-PEGYLATED LIPOSOMAL DOXORUBICIN OUTRUNS THE IMPACT OF A DOSE-DENSE REGIMEN IN VERY ELDERLY PATIENTS WITH AGRESSIVE B-CELL NON HODGKIN LYMPHOMA: AN ITALIAN MULTICENTRIC STUDY ON 129 PATIENTS

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Aims. The toxicity and efficacy of non-pegylated liposomal doxorubicin (NPLD) when substituted for conventional doxorubicin in the CHOP regimen (Doxorubicin, Cyclophosphamide, Vincristine, Prednisone) were prospectively evaluated in the treatment of 129 consecutive elderly patients with newly diagnosed aggressive B-cell non-Hodgkin lymphoma (NHL). **Methods.** Patients were split in 2 groups according to the Multidimensional Geriatric Assessment (MDGA). Thirty-nine patients with an Activities of Daily Living (ADL) = 6 were addressed to receive dose-dense R-COMP every 2 weeks (14), whereas 90 patients with an ADL < 6 were addressed to receive R-COMP every 3 weeks (21). The median age of patients was 74 years (range: 65-89). At baseline 94/129 (73%) patients had stage III-IV disease, and 60/129 (46%) patients had an intermediate or high risk International Prognostic Index score. Median performance status was WHO 1 (range 0-3) and median number of comorbities was 2 (range 1-6). The median left ventricular ejection fraction (LVEF) before starting chemotherapy was 59% (range 40-73). A total of 715 cycles of chemotherapy were administered (234 R-COMP 14 and 481 R-COMP 21). All these variables were comparable between the 2 groups (14 vs 21). **Results.** All patients were evaluable for response. The overall response rate was 89% (complete response 73%, partial response 16%). With a median follow-up of 24 months (range 2-20) as of January 2012, 87/129 patients (67%) are alive and disease free, whereas 32/129 (25%) are dead and 8/129 (6%) are alive with active disease. Log rank analysis showed high risk IPI (4/5; p=0.02) to be a variable predictive for shorter event-free survival (EFS), whereas again high risk IPI (4/5; p=0.04), age >70 years (p=0.009), advanced-stage disease (stage III-IV; p=0.01) and performance status 2-3 (p=0.003) were all predictive of shorter overall survival (OS). The Cox proportional hazards regression model identified age >70 years (p=0.03) and perfomance status 2-3 (p=0.02) as the variables with a negative impact on OS. Finally, no statistically significant difference in terms of response was observed within the two groups (14 vs 21).Toxicity was mainly hematological in both groups. Grade 3/4 neutropenia occurred in 11% and 22% of cycles in the R-COMP 14 and 21 groups respectively, with an incidence of febrile neutropenia of 3% and 8% respectively. The relative dose intensity for the regimens was 93% for the R-COMP 14 group and 90% for the R-COMP 21 group, respectively. Regarding cardiotoxicity, only 8/129 patients presented a grade II-IV WHO toxicity. **Conclusions.** Up to now, this is the largest series of elderly aggressive NHL patients treated with NPLD instead of conventional doxorubicin reported in literature. Our data strongly suggest that NPLD induces a high rate of long-lasting complete response (CR) in a population of elderly patients with relevant comorbidities. The overlapping rate of CR between the dose-dense R-COMP 14 group and the standard R-COMP 21 group suggest that the use of NPLD outruns the impact of a dose-dense regimen in a substantial proportion of very elderly patients, representing a therapeutic opportunity for a category of patients not suitable for a dose-dense treatment.

0237

ORAL LENALIDOMIDE PLUS 4 DOSES OF RITUXIMAB INDUCED PROLONGED REMISSIONS IN A COHORT OF PATIENTS WITH DLBCL AND GRADE 3 FOLLICULAR LYMPHOMA: A COMPLETED PHASE II CLINICAL TRIAL

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Background. The oral immunomodulatory agent lenalidomide has been shown to have significant single-agent activity with good tolerance in patients with diffuse large B-cell lymphoma (DLBCL). Its combination with monoclonal antibody rituximab might be more effective. After studying the combination in a phase I/II clinical trial in relapsed/refractory MCL patients (Wang et al, Lugano, 2011), we report our data from a Phase II clinical trial in relapsed/refractory DLBCL treated at the MTD. **Aims.** To evaluate the efficacy and safety of lenalidomide plus rituximab in patients with relapsed/refractory DLBCL. **Methods.** The study population includes patients with DLBCL (*de novo* or transformed) and Grade 3 follicular lymphoma. Patients received 20 mg oral lenalidomide daily on days 1-21 of every 28-day cycle and 375mg/m^2 intravenous rituximab weekly for 4 doses during cycle 1. Treatment continued until disease progression or severe toxicity. All patients signed an IRB-approved informed consent. The Kaplan-Meier method was used to calculate progression-free survival and response duration. **Results.** Forty five (45) patients with DLBCL were enrolled. Median number of prior therapies was 3 (range 1-4). Grade 3 and 4 toxicities, regardless of relationship to study therapy, included neutropenia (n = 24), thrombocytopenia (n=15), and lymphopenia (n=18). Other G3/4 events occurring in ≥ 2 patients include fatigue, rash, edema, embolism, elevated LFT, elevated LDH and electrolyte abnormalities. Four patients came off study because of toxicity and 12 required dose reductions. Overall response rate was 37% with a complete remission (CR + CRu) rate of 20% (CR rate of 16% and CRu rate of 4%) and partial remission (PR) rate of 13%. Median duration of response (CR/PR) was 10 months (95% CI). The median progression-free survival was 4 months (95% CI). After a median follow-up time of 16 months (7-41 months), the median overall survival was 11 months (95% CI). **Conclusions.** Oral lenalidomide plus rituximab was well tolerated and effective in patients with relapsed/refractory DLBCL and Grade 3 follicular lymphoma. Adding rituximab to lenalidomide might be superior to lenalidomide alone. A randomized clinical trial is needed for conclusive evidence.

0238

EVALUATION OF THE USE AND EFFICACY OF RITUXIMAB (R) MAINTENANCE REGIMENS IN US PATIENTS DIAGNOSED WITH FOLLICULAR LYMPHOMA (FL): DATA FROM THE NATIONAL LYMPHOCARE STUDY 2004-2007

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Background. Rituximab maintenance (RM) following induction therapy has been demonstrated to increase progression-free survival (PFS) in randomized trials in both first-line and relapsed settings (ECOG 1496, EORTC 20981, and PRIMA), and RM has frequently been used in clinical practice in the US and other countries. **Aims.** To examine the various approaches to RM used by practicing physicians, the correlates for use of RM, and the 'real-world' effectiveness of RM compared with observation (Obs) in patients with FL. **Methods.** The National LymphoCare Study (NLCS) is a multicenter, longitudinal, observational study of newly diagnosed FL patients enrolled from 2004 to 2007. All patients gave consent and were treated according to the enrolling physicians' preference; patients were followed for disease progression, retreatment, and overall survival (OS). Patients who achieved complete response, partial response, or stable disease following induction treatment with R-based therapy and had not initiated second-line therapy during the 215-day period following the date of last dose of induction therapy were categorized as Obs in this analysis, and those who started maintenance treatment during the 215-day period composed the

RM group.Predictors for receiving RM were identified using multivariate logistic regression analysis with backward stepwise selection. RM and Obs groups were compared for PFS, time to next treatment (TTNT), and OS using Cox proportional hazards models. **Results.** Of 2727 evaluable NLCS patients, 1204 completed R-based induction therapy and met all inclusion criteria for this analysis. 538 patients started RM treatment in the 215-day post-induction period and 666 patients were observed. The mean time to first RM dose was 4 months and mean duration of RM was 18 months. A total of 82% of RM patients completed their planned maintenance treatment regimen, 13% prematurely discontinued, and treatment was still ongoing for 5%. Predictors (p<0.05) for receiving RM over Obs were histology grade (1/2), stage (III/IV), geographic region (other than West), and center type (community practice). PFS and TTNT in the RM group were longer than those in the Obs group. With a median follow-up of 53 months after induction, OS was comparable between RM and Obs groups (HR=0.79; p=0.27). Adjusting for factors, including the Follicular Lymphoma International Prognostic Index, RM was associated with superior PFS (HR=0.69; p=0.003), longer TTNT (HR=0.73; p=0.03), but there was no difference in OS compared with Obs. The efficacy of RM treatment was not affected by the type of frontline regimen. At the time of analysis, 22% of RM patients had received second-line treatment; of these, 33% received R-monotherapy and 32% R-chemotherapy. In the Obs group, 25% received second-line treatment, 41% R-monotherapy, and 27% R-chemotherapy. The response rate for second-line treatment was similar between groups. **Conclusions.** NLCS data on physician-directed strategies following R-based induction provide useful information for comparing the effectiveness of RM and Obs in clinical practice. In this setting, RM treatment following R-based induction therapy produced significantly longer PFS and TTNT compared with Obs. Longer follow-up is required to determine whether this will produce differences in OS.

0239

IMPACT OF RELATIVE DOSE INTENSITY (RDI) IN TWO-WEEKLY DA-EDOCH14 COMBINED WITH RITUXIMAB ON SURVIVAL IN POOR PROGNOSIS DIFFUSE LARGE B-CELL LYMPHOMA

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Background. It has been pointed out that maintenance of relative dose intensities (RDI) is related to survival prognosis in patients with poor prognosis diffused large B-cell lymphoma (DLBCL); however, little information is available from routine clinical practice regarding how well dose intensity is maintained with modern infusional regimens such a as 3-weekly R-EPOCH. The aim of the present study was to explore a R-DA-EPOCH-like regimen, which is delivered every 2 weeks (two-weekly DA-EDOCH14-R) for the treatment of prognosis DLBCL and to compare the average RDI with that achieved by poor prognosis DLBCL patients treated on the prior 3-weekly DA-EPOCH-R protocol conducted by our group. **Patients and Methods.** Forty five patients (median 49.81 years old; range 18-70) with newly diagnosis of poor-prognosis DLBCL (age-adjusted IPI ³2) were enrolled: 18 patients were treated with 6 cycles of two-weekly DA-EDOCH14-R (from 10/08 to 01/12), and 27 with 6-8 cycles of standard 3-weekly DA-EPOCH-R (from 11/02 to 05/06). Two-weekly DA-EDOCH14-R was supported on day 5 by pegfilgrastim 6 mg s.c, and 3-weekly DA-EPOCH-R was supported by filgrastrim, at a dose of 5 µg/kg/day s.c from day 6 until day 11. The primary objective of our chart review was to calculate the delivered dose intensities of chemotherapy for these 45 poor-prognosis DLBCL patients, and determine if a higher RDI resulted in better responses to chemotherapy and 3-year survival rates. RDI was calculated as the delivered dose intensity (total dose delivered/total time of therapy) divided by standard dose intensity calculated for each regimen and compared to progression-free survival (PFS) and overal survival (OS). Multivariate recursive partitioning survival analysis was utilized. **Results.** The two-weekly DA-EDOCH14-R group had a higher incidence of extranodal sites involved ≥2 (60% vs. 26%, respectively; p=0.018), and high aa-IPI score (70% vs. 37%, respectively; p= 0.02) than the patients treated with the prior 3-weekly DA-EPOCH-R regimen. Table **1** lists the dose intensity by Treatment Group. The median RDI was significantly lower with two-weekly DA-EDOCH14-R regimen (0,97 vs 1,21, with R-EPOCH14 p < 0.001, respectively). Response rates showed a near-significant trend towards a better CR rates with the two-weekly DA-EDOCH14-R regimen (90% vs. 66.6% with the 3-weekly DA-EPOCH-R combination, p=0.09). At a median follow up of 29 months, the estimated 3-year progression-free and overall survival rates were higher in the two-weekly DA-EDOCH14-R group (95% vs. 74% in the 3-weekly DA-EPOCH-R group; p=0.08). **Conclusions.** Our data suggest shortening of intervals between standard doses with modern infusional regimens (two-weekly R-EDOCH14) in comparison with increasing doses and fixed intervals (3-weekly DA-EPOCH-R), seems to be more effective than dose intensification in patients with poor prognosis DLBCL. This preliminary data must be demonstrated in further studies.

Table 1.

	ADI	RDI	Cum dose mg/m2
R-EDOCH-14	127,12254	0,96852652	
Doxorubicine			248,139111
Etoposide			1201,70222
Cyclophosphamide			4513,83111
R-EPOCH	99,849819	1,20840809	
Doxorubicine			284,769053
Etoposide			1390,59259
Cyclophosphamide			5339,30864

0240

90YTTRIUM ZEVALIN FOLLOWED BY BEAM (Z-BEAM) AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR THE TREATMENT OF HIGH RISK RELAPSED/RESISTANT AGGRESSIVE NON HODGKIN'S LYMPHOMA (NHL)

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Background. High dose chemotherapy (HDC) and ASCT is actually considered an effective treatment for relapsed aggressive NHL. Standard dose Zevalin (0.4 mCi/kg) combined with conventional BEAM (Z-BEAM) is a promising conditioning regimen for the treatment of high risk relapsed/resistant NHL. **Aims.** We performed a single institution phase II study to determine the feasibility and to explore the possible sinergic effect of the addition of standard dose Zevalin to a BEAM high dose regimen in pts with high risk advanced stage NHL, with different histology, who relapsed or failed to respond after first line chemotherapy. A matched cohort analysis with a group of patients treated with standard BEAM without Zevalin was planned as secondary end point. **Methods.** Between October 2006 and May 2011 thirty pts were treated with Zevalin (day -14) followed by standard dose BEAM (day -7 to -1) and ASCT. Patients were included into the study and considered at high risk of failure if showed: progression or early relapse (<1 year) from previous therapy or multiple relapses. Standard dose DHAP or ICE with Rituximab were used as salvage chemotherapy and mobilizing regimen. **Results.** Clinical characteristics were as follows: 15 refractory and 15 early or multiple relapse; 8 grade I-II follicular, 17 PML/DLBCL, 3 MCL, 2 indolent non follicular; 7 stage II and 23 stage III-IV; 15 bone marrow involvement and 9 LDH level above normal. 14 patients received only one previous line of treatment and 16 were treated with 2 or more lines before Z-BEAM, all containing Rituximab. Only 5/30 patients received a reduced dose of 0.3 mCi/kg Zevalin because of low platelets counts. Response status before RIT was: 14 CR (47%), 10 PR (33%), 6 SD/PD (20%). At the end of treatment response status was: CR 19 (63%),PR 7(23%),PD 3 (10%) and TD 1 (4%). Median CD34+ cells infused was 7.26 10^6/kilograms (range 4.43-8.9). All pts engrafted with median time to platelet and neuthrophils count higher than $20x10^9$/L and $0.5x10^9$/L of 11 and 10 days respectively. With a median follow up of 27 months from salvage therapy 2-year progression free survival (PFS) is 63% and overall survival is 67%. 11 pts relapsed or progressed after Z-BEAM and 10 pts died, 8 of them for lymphoma, one because of lung Aspergillosis and H1N1 infection during BEAM and one for late encephalitis. We retrospectively compared this population with a matched-paired group of 21 pts treated with BEAM alone. No differences in clinical presentation at relapse were showed, apart the number of previous therapies: ≥2 only in 2 pts (9%) in BEAM group vs 16 pts (53%) in Z-BEAM group. ORR in BEAM group was 62% vs 86% in Z-BEAM group. A trend in favour of Z-BEAM was observed for PFS (63% vs 52%). **Conclusions.** In this group of pts with relapsed/resistant NHL at high risk of further relapse Z-BEAM+ASCT is able to achieve a good response with engraftment and toxicity not different from standard BEAM. 2-year PFS is promising, updated analysis with a longer follow-up is ongoing.

0241

THE PROGNOSTIC VALUE OF INTERIM POSITRON EMISSION TOMOGRAPHY BASED ON THE DEAUVILLE 5-POINT CRITERIA AFTER 3 OR 4 CYCLES OF RITUXIMAB PLUS CHEMOTHERAPY IN NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMA

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Background.Whole body positron emission tomography (PET) with ^{18}F-fluoro-2-deoxy-D-glucose is proposed as a reliable tool to predict treatment outcome in diffuse large B-cell lymphoma (DLBCL). However, there was a lack of uniform and reliable criteria for interpreting interim PET in DLBCL. In some studies, international harmonization project has been used for the interpretation of interim PET, but it was originally meant for assessment after termination of treatment. Recently, Deauville 5-point criteria has been recommended for interpretation of interim PET, but the prognostic data is limited. **Aim.** The aim of this study is to investigate the impact of interim PET using Deauville 5-point criteria on clinical outcomes in patients with DLBCL in terms of progression-free survival (PFS) and overall survival (OS). **Methods**. We consecutively enrolled 83 DLBCL patients treated with rituximab plus chemotherapy and performed an interim PET after 3 or 4 cycles of treatment in Chonbuk National University Hospital from Jan 2006 to Apr 2011. PET interpretation was done by two nuclear medicine specialists. **Results.** Total of 74 patients were included in this study. Nine were excluded because they had no initial PET data or had a single lesion excisioned before initial PET. The median age was 66 years (range 15-85) with 48 patients (64.9%) over 60 and 24 (32.4%) were female. The Ann Arbor stage was III in 16 (21.6%) and IV in 18 (24.3%) with nine involving bone marrow. ECOG performance status (PS) was 0 or 1 in 56 patients (75.7%), serum lactate dehydrogenase (LDH) level was elevated in 44 (59.5%) and B symptoms were present in 13 (17.6%). Bulky disease was identified in six patients (8.1%). Total of 16 patients (21.6%) were in the high risk International Prognostic Index (IPI).According to Deauville criteria 23 (31.1%) patients had 1 point, 25 (33.8%) had 2 points, 10 (13.5%) had 3 points, 15 (20.3%) had 4 points and one (1.3%) had 5 points. Those who had higher score than 4 (positive result in the Deauville criteria) were 16 (21.6%). After a median follow up of 22.4 month (range 6.0-69.1), the 3-year PFS and OS was 73.3±6.0% and 71.8±6.5%, respectively. The 3-year PFS and OS were significantly worse in patients having a positive interim PET (PFS, 30.1% vs 88.1%, *P*<0.001; OS, 26.4% vs 86.3%, *P*<0.001), respectively. The clinical variables associated with worse OS were as follows: age >60 years (*P*=0.004), Ann Arbor stage III or IV (*P*<0.001), ECOG PS 2 to 4 (*P*<0.001), presence of B symptom (*P*=0.002), elevated serum LDH level (*P*=0.026), extranodal involvement >2 (*P*=0.002), high IPI (*P*<0.001), positive interim PET (*P*<0.001). In multivariate analysis for OS, a positive interim PET (HR, 4.59; 95% CI, 1.33-15.7) and high IPI (HR, 5.83; 95% CI, 1.43-23.8) were independent prognostic factors for shortened OS. **Conclusions**. Interim PET analysis using Deauville 5-point scale in DLBCL is strongly predictive of survival outcomes. Further prospective studies are required to validate our results and optimize treatment strategies incorporating interim PET status to improve outcomes in patients with poor prognosis.

0242

SERUM CCL3 AND CCL4 LEVELS FUNCTION AS NOVEL PROGNOSTIC MARKERS IN DIFFUSE LARGE B CELL LYMPHOMA

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Background. B cell receptor (BCR) signaling is a critical pathway in various B cell malignancies, including diffuse large B cell lymphoma (DLBCL). Upon BCR stimulation, malignant B cells secrete the chemokine CCL3 and CCL4 (MIP-1α and β) to foster B cell interactions with accessory cells. CCL3 and CCL4 serum and plasma levels can readily be quantified using standard ELISAs, and are elevated in patients with chronic lymphocytic leukemia (CLL), where they function as an independent prognostic marker. Gene expression profiling in DLBCL revealed distinct signatures, such as the activated B cell (ABC) signature, which have prognostic implications. *SCYA3*, the gene coding for CCL3, is part of the ABC signature and associated with an inferior outcome. We therefore hypothesized that CCL3 and CCL4 serum levels may have prognostic impact in DLBCL. **Aims.** To determine the independent prognostic significance of serum CCL3 and CCL4 levels in untreated DLBCL patients. **Methods.** Serum samples from untreated DLBCL patients were analyzed for CCL3 and CCL4 levels by ELISA. Fisher's exact test was performed to evaluate the association between 2 categorical values. Spearman's correlation coefficiency was computed to correlate 2 continuous variables. Survival data were plotted according to Kaplan-Meier method, and group comparisons were made using the Log-rank test. Cox proportional hazard regression analysis was applied for multivariate analysis. **Results.** 102 patients from MD Anderson with untreated DLBCL were retrospectively analyzed. The median age of the cohort was 58 years old (range: 22 to 86) and median follow up duration was 14 months (1 to 33). 34 (33%) patients had an Ann Arbor Stage I or II, and 63 (67%) had III or IV disease. 65 patients (64%) had low International Prognostic Index (IPI) scores (0 to 2) and 37 (36%) patients had high scores (3 to 5). 95 (93%) patients received CHOP-like regimen with rituximab, and 7 patients (7%) received autologous stem cell transplantation as salvage therapy. Mean serum CCL3 and CCL4 levels were 49.9 ± 3.63 and 224 ± 17.4 pg/ml, respectively. High serum CCL3 levels (≥40) correlated with high IPI scores (p = 0.05), high LDH (p<0.01), and high β2 microglobulin (p<0.01). High serum CCL4 level (≥225) correlated with advanced Ann Arbor stages (p<0.01), high IPI scores (p<0.01), high LDH levels (p<0.01) and high β2 microglobulin (p<0.01). One year overall survival (OS) of the cohort was 91% (95% CI: 85-97), and 1 year progression free survival (PFS) was 81% (95% CI: 74-90). Log-rank test demonstrated that high CCL3 levels were associated with a significantly shorter PFS (p=0.029), and high CCL4 levels were associated with shorter OS (p<0.01) and PFS (p<0.01). Multivariate analysis revealed that high CCL4 level (HR: 3.2, p=0.03) and high IPI score (HR: 2.5, p=0.04) were significant and independent predictors for PFS. **Conclusions.** High serum CCL3 and CCL4 levels correlate with established prognostic markers and were associated with inferior outcome in DLBCL. CCL3 and CCL4 levels therefore should be considered for prognostication in DLBCL, particularly in the new era of BCR-targeting therapies.

Mantle cell lymphoma and others

0243

LYMPHOMA OCCURRING OVER THE AGE OF 90: CLINICAL PRESENTATION AND OUTCOME

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Background. The incidence of lymphoma increases with age. Meanwhile, life expectancy continues to progress (>4 years at the age of90 inFrance). Thus, lymphoma in very elderly patients is not uncommon, and its frequency should increase with time. Despite few reports on lymphoma in very elderly patients (>80 years), no data are available for patients over the age of 90, rendering the management of lymphoma in this particular population very challenging. **Aims.** The purpose of our study is to describe the clinical presentation, management and outcome of lymphoma occurring in patients over the age of 90. **Methods.** In a retrospective, multicenter study, we analyzed 143 patients with Hodgkin (HL, n=5) or Non-Hodgkin (NHL, n=138) lymphoma diagnosed at the age of 90 or above, between 1990 and 2011. **Results.** Among the 143 patients, the median age at diagnosis was 92 years (range=90-99), 59% of patients were female. Thirty-three percent of NHL were indolent and 67% were aggressive. The most frequent histologies were diffuse large B cell lymphoma (45%), marginal zone lymphoma (10%), and follicular lymphoma (8%). At the time of analysis, full clinical and biological characteristics were retrieved for 46 patients. At diagnosis, 83% (38/46) of patients were still living at their home, 9% (4/44) had dementia, 41% (19/46) had a low (<i*e.* ≤2) Charlson index, 57% (23/40) had a low (<i*e.* ≤1) performance status, and 28% (12/43) had B symptoms. Treatment choices were the following: no treatment or corticosteroids alone in 10 (22%) of cases, surgery or radiotherapy in 4 (8.5%) cases, monochemotherapy in 11 (24%) cases, polychemotherapy with anthracycline in 4 (9%) cases or without anthracycline in 17 (37%) cases. Median overall survival (OS) for the entire cohort (n=143) was 6.6 months. Patients with indolent lymphoma had a significantly longer survival than patients with aggressive lymphoma with a median OS of 14.6 months *versus* 5.4 months (p=0.002), respectively (Figure 1). Causes of death were analyzed for 33 patients so far: death was due to lymphoma in 15 (46%) cases, treatment in 4 (12%) cases, other cause in 10 (30%) cases, unknown cause in 4 (12%) cases.**Conclusions**Lymphomas diagnosed at the age of 90 or over are frequently aggressive. In this case, the prognosis is poor. The outcome of patients with indolent lymphoma is significantly better. The main cause of death is lymphoma progression whereas toxic deaths are less frequent. This raises the hypothesis that a more "intensive"therapeutic approach may be beneficial to a subset of patients in this population. Our results will be updated for the meeting presentation.

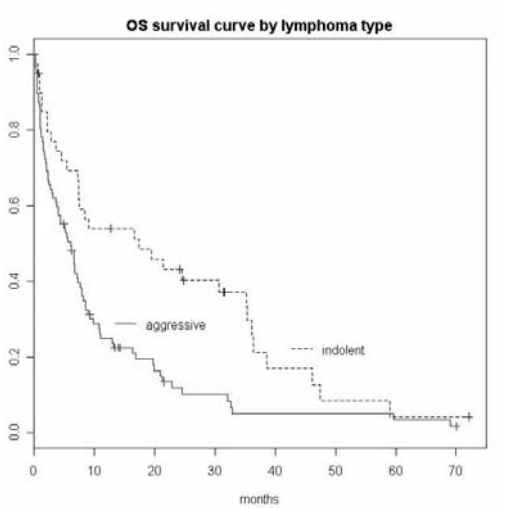

Figure 1. OS survival curve by lymphoma type.

0244

PROGNOSTIC VALUE OF PRE-TRANSPLANT POSITRON EMISSION TOMOGRAPHY USING FLUORINE 18-FLUORODEOXYGLUCOSE IN PATIENTS WITH NON HODHGKIN LYMPHOMA

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Background. Autologous stem cell transplantation (ASCT) is considered the standard salvage therapy for relapsed or refractory non-Hodgkin lymphoma (NHL). 18F-fluoro-deoxyglucose positron emission tomography (FDG-PET) was largely used to explore the predictive value in early evaluation of treatment and at the end of therapy. **Aims.** The aim of this study was to evaluate the role of FDG-PET performed before and after ASCT.Between January 2005 and August 2010 in the Transplant Unit of our centre were performed 82 autologous stem cell transplantation (ASCT) in patients with relapsed or refractory NHL. Fourthy eight out 82 transplanted patients performed an FDG-PET before ASCT and 44 performed FDG-PET after ASCT procedures. This is the group of patients analysed in the study. **Results.** The median age was 53 years (range 21-67 years); 32 were male and 16 female. At time of transplant 26 pts (54%) were in complete remission (CR) , 15 pts (31%) in partial remission (PR) and 7 pts (15%) presented a refractory disease. The FDG-PET before ASCT was negative in 29 pts (60%) and positive in 19 pts (40%). After a median period of observation of 29 months (range 1-83 months) the overall survival (OS) was 93% in the FDG-PET-negative group and 43% in the FDG-PET positive group (p: 0.002). After a median time of observation of 26 months (range 0-83 months), the progression free survival (PFS) was 88% and 42% (p : 0.001) respectively for pts with FDG-PET negative and positive (figure 1).After three months form ASCT 44 out 48 patients performed an FDG-PET for restaging, the FDG-PET was negative in 27 pts (61%) and positive in 17 pts (39%). The PFS was 88% in the FDG-PET-negative group and 28% in the FDG-PET positive group (p: 0.000) and the OS was 92% and 26% respectively in FDG-PET negative and FDG-PET positive scans (p:0.000). **Conclusions.** Our results confirm that in NHL a negative pre-transplant FDG-PET is associated with a better OS and PFS but a significant rate of patients with positive FDG-PET (42%) did not progress. Moreover our data confirm that in NHL the higher prognostic value of FDG-PET is associated with post-transplant or final result. Patients with a positive FDG-PET after transplantation is associated with a poor prognosis and should be considered for alternative treatments

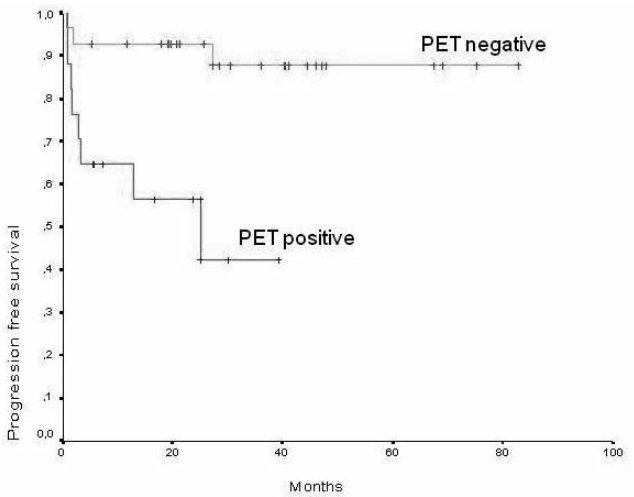

Figure 1.

0245

THREE DISTINCT SUBTYPES OF METHOTREXATE ASSOCIATED LYMPHOPROLIFERATIVE DISEASES IN PATIENTS WITH RHEUMATOID ARTHRITIS - A NEW CLINICAL CLASSIFICATION

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Background. Recent studies have highlighted the specific group of lymphoproliferative diseases (LPDs), other iatrogenic immunodeficiency-associated LPDs (OIIA-LPDs), especially in patients with rheumatoid arthritis(RA). Methotrexate (MTX) is one of the most potent drugs that can be mediated LPDs. Numerous attempts to uncover the mechanism of OIIA-LPDs-RA, the pathogenesis remains unclear. In addition, the strategy of therapeutic approach for OAIIA-LPDs and anti-RA treatment after LPD development is not delineated. **Aims.** To address the clinocopathogenesis of OIIA-LPDs-RA, we analyzed data on OIIA-LPDs-RA patients treated at our institution, with a focus on determining the preferred anti-RA medication protocol after LPD development. **Methods.** Data on 23 patients with RA who developed LPD and were treated at our institution were retrospectively analyzed. **Results.** Patients of MTX associated LPDs in patients with RA were categorized into 3 groups; 1) MTX-Regressive-LPDs, LPD regression occurred after withdrawal of MTX, 2) MTX-Persistent-LPDs, LPD persisted after MTX withdrawal, 3) Other-Mediated-LPDs, LPD developed only after MTX was discontinued (Figure 1).

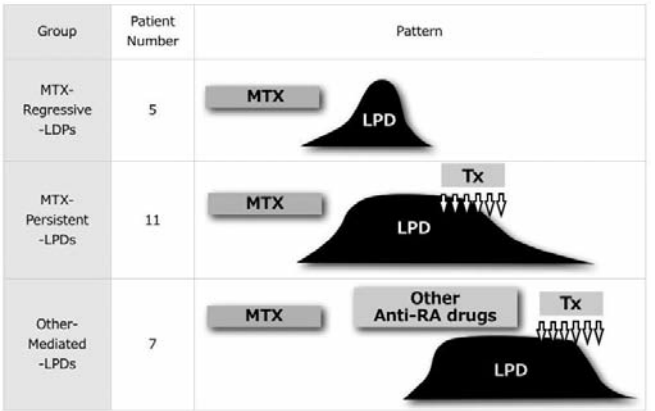

Figure 1. Three distinct subtypes of methotrexate associated lymphoproliferative

The patient's background of 3 groups showed a similarity regarding age, the duration of MTX treatment and the MTX total dose. Eleven of 23 patients were diagnosed with DLBCL, and various lymphomas including Hodgkin lymphoma and B/T- cell lymphomas were seen. The clinical stages and the laboratory data of LDH, CRP and sIL-2 in serum on the development of the LPDs in the MTX-Persistent-LPDs and Other Mediated-LPDs groups indicated significantly aggressive state of LPDs than those in MTX-Regressive-LPDs. The overall survival(OS) of all patients was 74% at 5 years, and those of 3 groups were 100%, 64%, and 60%, respectively. In 6 deceased patients, the delay in LPD detection was demonstrated in 4 patients because of the time lag in consulting with specialists from other hospitals. Some cases showed rarely LPD phenotypes: one case of DLBCL in MTX-Persistent-LPDs had a CD20 defect, and the other case of DLBCL in MTX-Persistent-LPDs was CD4 and CD30 positive. In 14 cases of karyotype analysis, 9 abnormal karyotype were detected. Of particular interest, the patient in the MTX-Regressive-LPDs showed a complicatedly abnormal karyotype that nonetheless vanished after MTX withdrawal. In the Other Mediated-LPDs group, MTX was not administrated at the LPDs development; azathioprine, bucillamine, salazosulfapyridine, tacrolimus and etanercept were given, and 3 drugs (tacrolimus, salazosulfapyridine, and etanercept) were given to the patients after LPD development; however, no patients showed LPDs regrowth. **Summary and Conclusions.** Of the data on 23 RA patients with LPDs, patients were categorized into three groups. The patients in MTX-regressive LPDs seemed a good prognosis, and OIIA-LPDs-RA itself appeared to have a better prognosis than other more common types of lymphomas. LPDs in OIIA-LPDs-RA might have a unique behavior. Various anti-RA drugs were given to the patients after developing LPDs, but no recurrent patients were documented.

0246

USE OF EPSTEIN-BARR VIRUS DNA MONITORING AS A SURROGATE MARKER OF DISEASE ACTIVITY IN POST TRANSPLANTATION LYMPHOPROLIFERATIVE DISEASE

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Background. Epstein-Barr virus (EBV) DNA levels from blood can be quantified using real-time polymerase chain reaction (RT-PCR). Monitoring of these levels after an organ transplant may allow for immune-modulation as a pre-emptive measure to reduce the incidence of post-transplant lymphoproliferative disease (PTLD), but evidence for doing this is mostly confined to the paediatric and bone marrow transplant populations. The utility of monitoring EBV DNA levels in adults who have received a solid organ transplant remains largely undefined. We present here our study using EBV DNA levels to follow disease activity once PTLD has been diagnosed. **Aims.** To evaluate the role of EBV DNA levels as a surrogate marker of disease activity in PTLD. **Methods.** This was a single center study at a major transplant hospital in Singapore. Consecutive patients with newly-diagnosed PTLD were prospectively followed with serial EBV viral load monitoring using a quantitative real-time polymerase chain reaction (PCR) targeted at the conserved region of EBNA-1 gene of EBV from whole-blood samples. These were paired and correlated with imaging studies done at diagnosis, and at assessment of response (by IWG criteria) as well as upon disease relapse. **Results.** Fifteen patients with newly-diagnosed PTLD were identified from 2005 - 2011, all were post-renal transplant. The median age was 62 years and median follow-up was 16 months. Histology was centrally reviewed for consistency according to WHO 2008 classification of PTLD. Thirteen patients had monomorphic PTLD (9 with diffuse large B-cell lymphoma [DLCL], 1 peripheral T-cell lymphoma [PTCL], 1 Burkitt lymphoma, 2 plasmablastic lymphoma) while 2 had CD20 negative polymorphic PTLD. Eight of fifteen patients had EBV-associated PTLD based on histological staining for EBER and LMP1. These 8 patients also had elevated EBV DNA titers at diagnosis. There were 3 other patients with detectable EBV titres at diagnosis despite being EBV-negative on tumor histology. The sensitivity of EBV PCR detection from blood in our PTLD group was 73.3%. Treatment which included the tapering of immune suppression, rituximab and CHOP chemotherapy was administered at the discretion of the treating physician. Eleven of fifteen patients achieved complete remission (CR), 2 had partial remissions, and another 2 had progressive disease as documented by CT or PET imaging. There were 2 relapses. All patients who had detectable EBV in blood at diagnosis and whom achieved a CR had negative titres at completion of therapy. A diminution of EBV titers correlated with partial responses at interim staging. In the 2 patients who suffered relapse, one (who was in CR) had reappearance of EBV in blood while another (in PR) had a rise in EBV titres at relapse.**Summary and Conclusion.** In our patients with a positive EBV DNA at diagnosis, monitoring these levels accurately predicted clinical response as well as relapse in PTLD. Our numbers are small as this is a single center experience and PTLD is a relatively rare disease in adults. Larger studies are needed to confirm our findings.

0247

THE ADDITION OF BORTEZOMIB TO STANDARD DOSE CHOP CHEMOTHERAPY SIGNIFICANTLY IMPROVES SURVIVAL IN RELAPSED MANTLE CELL LYMPHOMA

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Background. Bortezomib has demonstrable activity in mantle cell lymphoma and is licensed in the US for this indication based on a single arm phase II tri-

al. Randomised trials with Bortezomib in this disease have not been published although a European registration study is on-going. We report the interim analysis of a UK NCRN randomised trial which subsequently lead to the discontinuation of the study. **Aims.** To explore the addition of Bortezomib to CHOP chemotherapy in relapsed MCL. **Methods.** This was a parallel phase II randomised trial of CHOP chemotherapy versus CHOP with Bortezomib (VCHOP) in patients at first relapse with MCL. The Bortezomib was administered at 1.6 mg/m^2 on days 1 and 8 of standard dose CHOP chemotherapy. Full dose vincristine was given in both arms. There was no stratification. The primary end point was overall response rate with secondary end points of PFS, OS and toxicity and tolerability of the 2 therapies. Rituximab was not used as this was not widely available in the UK when the trial was initiated. **Results.** 46 patients have been treated, 23 in each arm. The arms were well balanced (CHOP v VCHOP) by median age, 71yrs v 69yrs, median WCC 5.1 v 6.1, median time from diagnosis 19.7 v 24.7 months. Almost all patients had stage IV disease with WHO performance status 0-1. There were more men in the CHOP arm 91% v 65% and more patients has received prior rituximab in the CHOP arm 44% v 17%. Almost all of the patients had previously received fludarabine and cyclophosphamide with or without Rituximab as their initial therapy.The overall response rates were 43.4% (22% CR) for CHOP and 82.6% (35% CR) for VCHOP p=0.03. At a median follow up of 24.2 months the PFS is 8.2 months CHOP and 15.7 months VCHOP (p=0.07) with an overall median survival of 16.4 months CHOP and 36.1 months VCHOP (p=0.026, estimated hazard ratio is 0.4)There was no significant difference in severe sensory neuropathy (Gd III/IV) between the two arms, 3 cases with VCHOP compared with 2 with CHOP. However there was more Gd I/II sensory neuropathy with VCHOP, 10 v 3 cases and in addition there were 2 cases of Gd I/II motor neuropathy seen in the Bortezomib arm.There was more Gd III/IV haematotoxicity in the VCHOP arm; anaemia 8 v 4 cases, neutropenia 13 v 11 cases and thrombocytopenia 9 v 8 cases. There was no difference in other observed toxicity apart from 2 DVT's in the VCHOP arm.Summary: The addition of Bortezomib to standard CHOP chemotherapy significantly increases response rates and overall survival in relapsed MCL with modest additional toxicity.

0248

CONVENTIONAL CYTOGENETIC (CC) IN MANTLE CELL LYMPHOMA (MCL) IS A STRONG PROGNOSTIC TOOL INDEPENDENT OF THE MCL INTERNATIONAL PROGNOSTIC INDEX (MIPI) AND USEFUL TO ISOLATE INDOLENT MCL AT DIAGNOSIS

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Background. MCL is characterized by the t(11;14)(q13;q32) and has an aggressive evolution with a median survival of 5 years. However, few patients, hardly recognizable at diagnosis, do have an "indolent"disease (iMCL) with a long survival without intensive therapy. To date, MIPI, blastoid variant and proliferation index (ki67) are commonly used as prognostic factors. Many studies highlight the role of additional genetics abnormalities (AA), but these abnormalities are not routinely tested and do not yet influence treatment decision. **Aims.** Therefore, we evaluated, in a cohort of t(11;14) MCL, the prognostic impact of AA detected by CC and their relationship with MIPI. **Methods.** All MCL cases diagnosed according to WHO criteria, between 1995 to 2011, were retrospectively selected, from 4 institutions, on the basis of an informative karyotype with t(11;14) at diagnosis. To isolate iMCL at diagnosis, we derived an "indolent score", based on clinical and cytogenetic characteristics that were selected as jointly associated with survival without intensive treatment (TFS) on a multivariate Cox model. Strength of association was assessed by hazard ratio (HR). **Results.** A total of 125MCL patients were included in this study. They were mostly male median age of 64 years; 87% displayed a stage IV disease, 68% a nodal presentation (lymph nodes >1cm), 55% an extranodal disease, 53% a spleen enlargement, 19% a blastoid variant and 48% a Ki67>30% (27/56 evaluated patients). The MIPI distribution was 33%, 27%, 40% for low, intermediate and high score respectively. CC analysis showed a total of 637 AA (range:0-37); 20% had isolated t(11;14), 16% one AA, 11% two, 53% a complex karyotype (CK), 6% a near tetraploid karyotype. Losses were more frequent than gains. Most frequent AA were del13q (24.8%), del9p (20.4%), del9q (19.2%), del17p (18.4%), del6q (19.2%), del1p (17.6%), del10p (12.8%), del10q (12.8%), del11q (8.8%) and tri3q (9.6%). Median follow-up was 35 months, median survival estimation 74 months. In a multivariable Cox model, high MIPI (HR=3.8, 95%IC:1.99-7.39, p<0.0001), CK (HR=2.37, 95%IC:1.17-4.79, p=0.017) and blastoid variant (HR=2.58, 95%IC:1.30-5.12, p=0.007) were independltly associated with a shortened survival.At time of analysis, among the 125 patients, 14 were alive without intensive treatment, 111 either received intensive therapy (n=106) or died before any intensive therapy (n=5); median TFS was 39 days (95%CI:31-59). Based on a multivariable Cox model, 4 variables were selected as associated with the outcome, namely spleen enlargement (HR=1.7, 95%CI:1.07-2.56), nodal presentation (HR=3.8, 95%CI:2.25-6.30), extranodal involvement (HR=2.4, 95%CI:1.57-3.76) and CK (HR=2.10, 95%CI:1.29-3.28). This allowed distinguishing two main groups of patients: "indolent"patients were defined as those who had no more than one risk factor excluding nodal presentation (n=18, whose median TFS was 53 months), and the remainders (median TFS: 1 month). **Conclusion.** In this multicentric cohort of t(11;14)MCL, complex karyotype, high MIPI and blastoid variant are 3 independent prognostic factors. With an "indolent score", relying on clinical and cytogenetic characteristics, we could discriminate iMCL at diagnosis. Therefore, besides being a tool for diagnosis, CC could be useful for the treatment decision in MCL.

0249

THE ADDITION OF STEM CELL TRANSPLANTATION FOLLOWING INDUCTION CHEMOTHERAPY IMPROVES OVERALL SURVIVAL IN MANTLE CELL LYMPHOMA PATIENTS WHO ACHIEVE A COMPLETE RESPONSE

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Background. The role of high-dose chemotherapy and stem cell transplantation (SCT) in first complete remission (CR1) for patients with mantle cell lymphoma (MCL) remains controversial. We evaluated 135 patients with MCL who were treated with anthracycline containing regimens or HyperCVAD/alternating with methotrexate/cytarabine (M/A) and obtained a CR. Based on physician preference, some patients subsequently received high dose chemotherapy and SCT. **Aims.** The aims of the analysis were to describe the characteristics of MCL patients who obtained a CR with induction chemotherapy. The risk of progression, progression-free survival (PFS), and overall survival (OS) were evaluated in patients who received induction chemotherapy +/- high dose chemotherapy with SCT. **Methods.** A total of 162 patients with newly diagnosed MCL were treated between 2000 and 2010. Of those patients, 135 obtained a first CR with induction chemotherapy. Patients were divided into two groups - Group 1 received an anthracycline containing chemotherapy (ex: CHOP +/- Rituximab (R)(N=62) and Group 2 received HyperCVAD/-M/A (+/- R) (N=73). Within each group, patients were evaluated for PFS, OS, and a multivariate analysis for risk of progression, treatment failure and death.

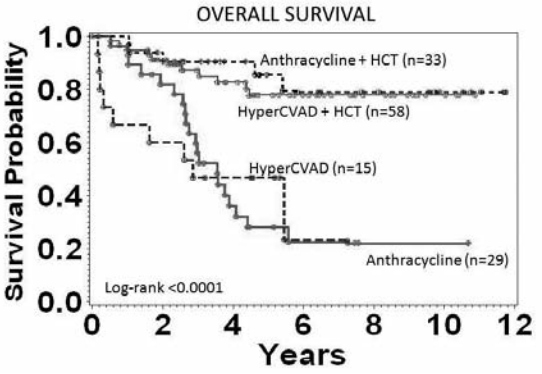

Figure 1. Overall survival.

Results. With a median follow up of 5 years (range 1-12 years), Group 1 consisted of 29 patients who received an anthracycline containing regimen alone (median age 70 years -[range 55-86]) while 33 patients received an anthracycline + SCT (median age 58 [range 39-75]). The 5-year OS for patients receiving anthracycline alone was 28% (95% CI, 13-46%) compared to 86% (95% CI, 65-94) for those patients receiving an anthracycline regimen followed by SCT in CR1 (p<0.001). Group 2 patients had a median follow up of 6 years (range 2-13 years), 15 patients received HyperCVAD- M/A alone (median age 53 years [range 32-75]) and 58 patients received HyperCVAD -M/A followed by SCT (median age 56 years [range 35-70]). The 5-year OS for patients receiving HyperCVAD- M/A alone was 47% (95% CI, 21-69%) compared to 78% (95% CI, 63-87%) for those receiving HyperCVAD-M/A with ASCT (p=0.03). In

multivariate analysis, both groups had a decreased hazard risk of death with the addition of SCT (anthracycline group, p=0.02) and (HyperCVAD- M/A, p=0.001). The only other significant covariate for the risk of death was age (p=0.01). **Conclusions.** For patients with MCL that achieve a complete remission with either an anthracycline or HyperCVAD- M/A, the use of high dose chemotherapy and SCT improved the overall survival when used as a consolidation therapy.

0250

TEMSIROLIMUS IN PATIENTS WITH RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA: DOES PROGNOSTIC RISK AFFECT OUTCOMES?

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Background. Temsirolimus (TEM) is a selective inhibitor of mammalian target of rapamycin approved for treatment of relapsed or refractory mantle cell lymphoma (MCL) in Europe, Israel, Serbia, Australia, Hong Kong, Taiwan, and the Philippines. In the pivotal phase III trial (*J Clin Oncol* 2009;27:3822-9), TEM 175/75 (175 mg for first 3 weeks then 75 mg weekly) significantly prolonged progression-free survival (PFS) versus investigator's choice of therapy (INV) (4.8 vs 1.9 months, respectively; hazard ratio [HR]=0.44; *P*=.0009). TEM 175/25 (175 mg for first 3 weeks then 25 mg weekly) also showed longer PFS versus INV, but this difference was not significant (3.4 vs 1.9 months; respectively; HR=0.65; *P*=.06). However, at the time of the trial, patients were not categorized according to prognostic risk group at baseline. More recently, the simplified MCL International Prognostic Index (MIPI) was validated as a good predictor of survival (*Blood* 2008;111:558-65; *Blood* 2010;115:1530-1533). **Aims.** In this post hoc study, patients were retrospectively assigned simplified MIPI prognostic scores and outcomes were analyzed according to risk category. **Methods.** All patients (N=162) were classified (at study entry) as low, intermediate, or high risk using simplified MIPI scores, which are based on 4 independent prognostic markers: age, Eastern Cooperative Oncology Group performance status, lactate dehydrogenase level, and white blood cell count. Median PFS and overall survival (OS) were calculated using Kaplan-Meier estimates; significance of the treatment effect was indicated by log-rank P values ≤.05. The phase III study was not powered for this analysis, and statistical analyses shown are for explanatory purposes. **Results.** Distribution was relatively even across MIPI categories (55 patients low, 59 intermediate, 48 high). MIPI distributions in both TEM arms were: 175/75 (n=54: 28% low, 43% intermediate, 30% high); 175/25 (n=54: 28% low, 33% intermediate, 39% high). Relative to the TEM arms, the INV arm (n=54) had a higher proportion of low-risk patients (46% low; 33% intermediate; 20% high). Treatment with TEM 175/75 resulted in a significant improvement in median PFS (independent assessment) versus INV in high-risk patients (*P*=.003); trends toward improvement were observed for intermediate-risk and low-risk patients (*P*=.06 in each group). By investigator assessment, TEM 175/75 significantly improved median PFS versus INV by 7.9 months in the low-risk category (*P*=.0007) and by 2.8 months (*P*=.06) and 1.1 month *(P*=.001) in the intermediate-risk and high-risk categories, respectively. In all risk categories, more patients who received TEM 175/75 mg achieved stable disease compared with INV. In this study, simplified MIPI was a good predictor of OS in patients with relapsed/refractory disease. A trend toward longer OS was observed in low-risk patients treated with TEM 175/75 versus INV (*P*=.05). In both the TEM 175/75 and INV groups, grade 3/4 anemia, thrombocytopenia, and infection occurred more commonly in high-risk than low-risk patients. In the low-risk category, a higher incidence of grade 3/4 thrombocytopenia and anemia was observed with TEM 175/75 versus INV. **Conclusions.** Temsirolimus is effective for all risk groups of patients with relapsed/refractory MCL. Low-risk patients may potentially derive the greatest clinical benefit.

0251

ULTRA-RAPID 60 MINUTE INFUSION OF RITUXIMAB IN NON-HODGKIN'S LYMPHOMA -IMPLICATIONS FOR PRACTICE AND RESOURCES

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Background. Rituximab is an anti-CD20 monoclonal-antibody and core component of non-Hodgkin's lymphoma (NHL) therapy. It is frequently given in combination with cytotoxic chemotherapy and as a maintenance therapy in follicular lymphoma.Infusion related reactions are a common complication of rituximab therapy, particularly on the first infusion where it is recommended to be given following an escalating rate schedule. Subsequent infusions are given more quickly with an average infusion time of 2 ½ hours. There is increasing evidence to support the administration of subsequent doses by 'rapid infusion' over 90 minutes, but evidence for 60 minute infusion safety is more limited. **Aim.** This audit aims to establish the safety of 60 minute infusion of non-first dose rituximab in patients with NHL, and to evaluate the impact that this may have on manpower and resources. **Methods**.100 60 minute rituximab infusions in 33 patients with NHL were audited both prospectively and retrospectively. First doses were given using the recommended escalating rate schedule, but subsequent doses were infused over 60 minutes. Data collected included sex, diagnosis, chemotherapy regimen, incidence and nature of adverse infusion reaction on cycle 1 and incidence of infusion reactions on subsequent cycles. **Results.** 100 60 infusions were audited in 33 individual patients, 13 female and 20 male. Lymphomas were 20 follicular, nine diffuse large B cell, two marginal zone, one T-cell rich B-cell and one high grade B-cell not otherwise specified. Rituximab was given as part of R-CHOP (15) R-CVP (eight) R-FC (one), R-Chlorambucil (one) and as single agent maintenance (nine). One patient had two treatment lines. All patients received 10mg IV chlorphenamine and 1g oral paracetamol as standard but steroids were only given when as part of the planned chemotherapy schedule (80 infusions = 80%). Seven (21%) patients experienced infusion related reactions on cycle 1, all but one were of NCI grade 1-2. One (3%) patient experienced NCI grade 3 reaction (on R-CHOP). On subsequent cycles only this patient (3%) experienced an adverse infusion reaction (grade 2, flushing, urticaria and temperature of 38.c). This resolved following administration of IV hydrocortisone and the infusion completed safely over 90 minutes thereafter. **Conclusions.** In NHL, non first dose rituximab can be safely infused in 60 minutes without significant increase in infusion related adverse reactions. Only one patient failed to tolerate the 60 minute infusion, also having experienced the only identified grade 3 reaction on cycle 1. In one year, with a catchment population of 600,000, our service gave 535 doses of rituximab that would be eligible for 60 minute infusion. Considering an average dose of 700mg infused over 150 minutes, we project that the ability to administer 97% of these infusions in 60 minutes would provide a saving of up to 778.5 day-case chair hours and nursing hours per annum. Such significant savings promote more efficient use of day-case treatment resources and of the nursing workforce in day-case areas that are experiencing increasing pressures and demands.

0252

WITHDRAWN

0253

BENDAMUSTINE IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA: RETROSPECTIVE ANALYSIS OF THE SPANISH EXPERIENCE

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Background. Patients with Mantle cell lymphoma (MCL) have an adverse outcome after relapse due to refractory disease with conventional treatments. Bendamustine (B), a nitrogen mustard compound chemically related to alkylating agents, has demonstrated high efficacy with a low toxicity profile in reported clinical trials. **Aims.** To analyze the Spanish experience in patients with relapsed/refractory MCL treated with Bendamustine. **Methods.** Retrospective analysis of spanish experience using Bendamustine alone or in combination in the relapse setting. This study was approved by local ethical committees. **Results.** Forty-three patients have been registered from May to December 2011. *Patients'characteristics*: 67% male, median age 65 years old (range 41-88), 77% ECOG≤ 1, 77% Ann Arbor stage IV, 35% high risk MIPI and 12% blastic variant. Previous regimens were CHOP or CHOP like ± R in 41.5%, Hyper-CVAD/MtxAraC ± R in 39%, R-CVP in 10% and other regimens in 9.5%. Median number of previous treatments were 2.5 (range 1-6), 93% patients had received prior Rituximab and 73% had chemosensitive disease to the last treatment. Bendamustine regimen was R-B (R-375mg/m^2 D1, B-90 mg/m^2 D1-2) in 74% patients, R-B with B-70 mg/m^2 in 9%, B alone in 5%, R-B-Bortezomib in 2% and R-B plus consolidation with stem cell transplant or Y^{90}Ibritumomab-tiuxetan in 9%. Median number of cycles was 4.62 (range 1-8). G- CSF support was administered in 46% of cycles. *Response*: Overall response rate was 80%, with 46% CR & uCR and 34% PR. *Survival:* Median progression free survival (PFS) was 18 months (95% CI: 7.6-28.4), data that compares favourably with patients' PFS to previous therapy (12 months, 95% CI: 9.4-14.5). Median PFS for patients who achieved CR/uCR was 35.9 months (95% CI: 19.3-52.7) versus 8.4 months in patients with PR (95% CI: 4.7-12.2). With a median follow-up for surviving patients of 14 months since Bendamustine treatment, the estimated OS at 3 years is 48% (+ SD 11%). In the whole series median overall survival from diagnosis is 11.8 years (range: 5.3-18.3 years) without plateau. *Toxicity:* No treatment related mortality has been described. Over 194 cycles, there were 9 hospitalizations due to febrile neutropenia. No tumoral lysis syndrome has been reported. **Conclusions.** Our results with Bendamustine, alone or in combination, confirm the efficacy of this agent in the treatment of MCL, even in the setting of unselected patients previously exposed to multiple therapies including Rituximab.

0254

INTERIM FDG-PET/CT DOES NOT PREDICT OUTCOME IN NEWLY DIAGNOSED MANTLE CELL LYMPHOMA PATIENTS TREATED WITH CHEMOTHERAPY ALONE OR ASCT

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Background. Mantle cell lymphoma (MCL) is a rare disease, accounting for about 6% of all new lymphoma patients per annum. It is considered an incurable disease; nevertheless, a small proportion of patients may have long term remission after upfront autologous stem cell transplantation (ASCT). FDG-PET/CT scans are often used to evaluate patients with MCL at diagnosis, interim and end of therapy, although the impact of PET results on their outcome is not well established. **Aims.** In this study we looked for correlation between interim PET results and outcome in a retrospective cohort of MCL patients. We also attempted to identify the predictive value of interim and pre-ASCT PET in a subgroup of MCL patients who underwent upfront consolidation with high-dose chemotherapy and ASCT. **Methods.** We retrospectively reviewed the demographics, clinical features and outcome of 58 consecutive MCL patients who were treated at three medical centers between 1998 and 2011. PET/CT scans, performed after 3-4 cycles and at the end of first line chemotherapy (prior to ASCT), were reviewed and correlated with response and long-term outcome. Scans were scored as positive or negative based only on visual assessment, according to the revised response criteria adopted in 2007. **Results.** Fifty eight patients were included. Forty nine were male and 9 female. Median age at diagnosis was 59 years (yrs) (range 41-88). All but three patients had stage 3/4 disease. Forty four (76%) patients received RCHOP or RCHOP-like chemotherapy for induction, and 23 (40%) patients were treated by BEAM followed by ASCT as part of their upfront consolidation. After a median follow-up of 3.3 yrs (0.67-13.1) the estimated overall survival (OS) and progression free survival (PFS) at 3 yrs for the entire cohort were 84% and 48 %, respectively. In the subgroup of patients (n=23) who received ASCT consolidation, the Kaplan-Meier analysis demonstrated 82% OS and 68% PFS at 3 yrs. MIPI group (low, intermediate or high) predicted OS and PFS ($p<0.05$). Interim PET was performed in 51/58 patients. The interim-PET positive (n=31) and PET-negative (n=20) patients did not differ in gender, MIPI, stage or presence of B symptoms. However, older age (≥60) was significantly more common in the interim-PET positive group (p=0.02). The estimated 3 yr OS for interim-PET negative and interim-PET positive patients were 85% and 90%, respectively (p=NS). The 3 yr PFS for interim-PET negative and positive were 57% and 44%, respectively (p=NS). A subset analysis of the patients who received consolidation with upfront ASCT showed that neither OS at 3 years (75% for PET negative vs. 90% for PET positive) nor PFS at 3 years (80% for PET negative vs. 66% for PET positive) were predicted by interim PET results. Similarly, the pre-transplant PET results did not correlate with the outcome in this subgroup of patients. **Conclusions.** We conclude that negative interim PET is more likely to be found in younger MCL patients. However, interim FDG-PET/CT results do not predict OS and PFS. In patients, who received consolidation with upfront transplant, neither interim nor pre-transplant PET results predict outcome.

0255

THE PROGNOSTIC ROLE OF MANTLE CELL INTERNATIONAL PROGNOSTIC INDEX (MIPI) IN PATIENTS TREATED WITH RITUXIMAB AND HIGH DOSE CHEMOTHERAPY PLUS AUTOLOGOUS STEM CELL TRANSPLANT

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Background. Mantle cell lymphoma (MCL) had dismal prognosis, with continuous relapses. In young patients promising results were obtained with first line chemoimmunotherapy followed by high-dose cytarabine containing regimens and high dose chemotherapy plus autologous stem cell transplantation (HDC

plus ASCT). The Mantle Cell Lymphoma International Prognostic Index (MIPI) had an established role in predicting survival in MCL patients treated with conventional chemotherapy. **Aims.** of the study was to investigate the prognostic role of MIPI in a retrospective group of MCL patients treated with Rituximab-chemotherapy and HDC plus ASCT. **Methods.** Between 1999 and 2009, 105 untreated MCL patients <70 years were consecutively treated with Rituximab-chemotherapy followed by high-dose cytarabine and HDC plus ASCT. Histology was centrally reviewed and Ki-67 evaluation was performed. MIPI and MIPI-biological were calculated according to simplified formulas according to Hoster 2008. International Prognostic Index (IPI) was calculated and considered as standard prognostic index. Overall Survival (OS) curves were estimated and stratified by MIPI. Differences between curves were tested using the 2-tailed log-rank test. In order to quantify the predictive discrimination of MIPI, MIPI-biological and IPI scores, a Cox's model analysis and univariate logistic models (with death and failure event as binary outcomes) were fitted and the area under the receiver operating characteristic (ROC) curves (c-index) was estimated. **Results.** Clinical characteristics were: median age 61 (34-70) years, 87% stage IV, 84% with bone marrow involvement, 22% with blastoid variant. Patients at high-risk (HR) were 47% according to MIPI, 42% according to MIPI-biological and 44% according to IPI. With a median follow-up of 59 months, 4-year Overall Survival by increasing MIPI risk was 92% for low risk (95% Confidence Interval: 85%-100%), 71% for intermediate risk (CI: 47%-100%) and 63% for high risk (CI: 44%-89%); log-rank test for Overall Survival was: p-value = 0.0001 (Figure 1). In order to quantify the predictive discrimination of MIPI, MIPI-biological and IPI scores, c-index and Cox-index for death event and for failure event were calculated. Logistic c-index for Overall Survival was as follows: MIPI 71.2%, MIPI-biological 62.2%, IPI 72.4%; for Progression Free Survival was: MIPI 63.1%, MIPI-biological 59.5%, IPI 68.8%. Cox-index for death event was: MIPI 73.2%, MIPI-biological 66.8%, IPI 71.7%; cox-index for failure event was: MIPI 68.7%, MIPI-biological 64.5%, IPI 66.7%. **Conclusions.** MIPI score should be a good predictor of death event in patients affected by MCL and treated at diagnosis with Rituximab-chemotherapy followed by HDC plus ASCT. In this retrospective series of patients MIPI had a clear role in predicting survival; the real role of MIPI as predictor of death should be validate in prospective series of patients treated with Rituximab-chemotherapy followed by HDC plus ASCT.

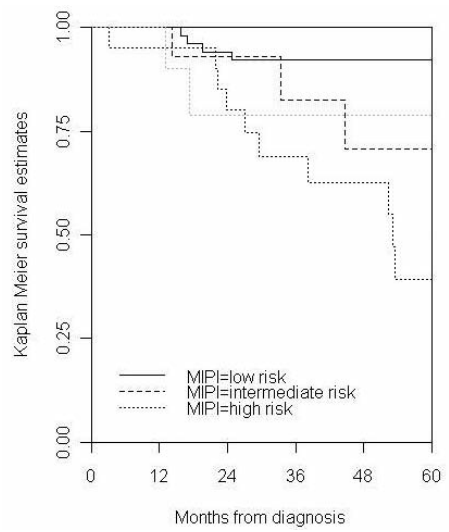

Figure 1.

0256

FLUDARABINE-BASED COMBINATION CHEMOTHERAPY IN RELAPSED MANTLE CELL LYMPHOMA - THE EXPERIENCE OF A SINGLE CENTER

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Background. Mantle cell lymphoma (MCL) is a rare subtype of lymphoma associated in most cases with poor prognosis and progressive resistance to chemotherapy. Treatment of relapses depends on regimens used as first line but the efficacy is usually limited. Fludarabine-based regimens have shown to be effective, although associated with significant hematological toxicities. **Aims.** To evaluate the clinical benefit and toxicities of fludarabine-containing regimens in relapsed MCL patients. **Methods.** A retrospective analysis of MCL patients diagnosed in our institution between 1999 and 2011 was conducted. The diagnosis was established by histopathological criteria, including cyclin D1 positivity, and/or detection of t(11;14). Response was classified according to *Cheson et al.* revised criteria. Toxicity was graded according to the common toxicity criteria, version 3.0. **Results.** Ninety-two MCL patients were identified in this period. Among the 57 who relapsed, 34 were salvaged with fludarabine based-regimens (56% associated with antracyclins and 65% with rituximab). The remaining were either candidates to high-dose chemotherapy and autologous stem cell transplant or unsuitable for fludarabine due to comorbidities or poor performance status. There were no significant differences regarding age, gender, stage, MIPI score, histological subtype and β2-microglobulin, between patients who received fludarabine-based regimens and the overall MCL patient population. In most cases (70.6%; n=24) fludarabine-based regimens were used as second line treatment (1-5 prior treatments), at a median time after diagnosis of 27 months; the majority (85.3%, n=29) of these patients received CHOP-like regimens as first line, associated with rituximab in 22 cases. 31 out of 34 fludarabine-treated patients were evaluable for response, with an overall response rate of 51.6% and complete remission rate of 19.4%. Median duration of response was 14 months and the median survival of responding patients was 21.5 months; however, the median time to next treatment for the whole population was only 3 months. With a median follow up of living patients of 7 months after starting fludarabine, the median survival was 8.5 months. Most patients (70%) experienced hematological toxicity, 62% of those graded 3/4. Febrile neutropenia occurred in 29% of patients (n=10/34), 6 requiring hospital admission. Treatment was prematurely discontinued in 45.5% (n=15/34) of cases, 9 due to toxicity and 6 due to disease progression with only 55% (n=18/33) patients receiving the intended treatment. **Conclusions.** In this small series of relapsed MCL patients, fludarabine-based regimens were associated with reasonable response rates and response duration. However, this did not translate in significant survival benefits for the whole population and was achieved at the expense of significant toxicities which determined therapy discontinuation in nearly half of patients, limiting the potential benefits to a selected population. This non-comparative study raises the question whether the benefit outweighs the potential risks of fludarabine therapies for relapsed disease, as it was recently shown for first line patients, when other active agents are being investigated in this setting.

0257

RELAPSED OR REFRACTORY SYSTEMIC ANAPLASTIC LARGE-CELL LYMPHOMA (ALCL) - A BURDEN OF ILLNESS ANALYSIS

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Background. Systemic ALCL (sALCL) is a subtype of peripheral T-cell lymphoma (PTCL), a subset of the T-cell lymphomas, which in turn are a subtype of non-Hodgkin's lymphoma (NHL). sALCL accounts for approximately 3% of NHL cases. Between 25-45% of sALCL patients relapse following or are refractory to first-line therapy; there is no standard of care in these relapsed or refractory (rel/ref) patients. As a result of the small patient numbers, research into the epidemiology and disease burden of rel/ref sALCL is very limited. **Aims.** To describe the burden of sALCL, in terms of its epidemiology and humanistic burden, and to estimate current costs of treatment of patients with rel/ref sALCL. **Methods.** A literature search for sALCL epidemiology data was conducted. A systematic literature review of the clinical effectiveness and safety of available therapies in rel/ref sALCL was conducted in Medline/Medline in Process, Embase and the Cochrane Library, and targeted searches for treatment guidelines were conducted. To estimate the costs of rel/ref sALCL, a treatment pathway in patients with rel/ref sALCL in the UK was built based on guidelines recommendations and clinical experts' opinions. An annual cost per patient was estimated, including costs of drug acquisition and administration, monitoring costs, and costs relating to stem cell transplant (SCT), adverse events (AEs), and long-term care. **Results.** sALCL is a rare disease. There are an estimated 1,500 new sALCL cases per year in the EU5, of which approximately 285 are in the UK. Of these, approximately 86 were estimated to be incident rel/ref cases. The systematic review of treatments for rel/ref sALCL showed that there is no standard of care. Among novel therapies recommended for PTCL in the US National Comprehensive Cancer Network Clinical Practice Guidelines for NHL are pralatrexate, romidepsin, and, more recently, brentuximab vedotin (systemic ALCL only). The treatment pathway for sALCL at relapse includes chemotherapy regimens such as gemcitabine, GEM-P (gemcitabine, cisplatin, methylprednisone), ICE (ifosfamide, carboplatin, etoposide), G-CVP (gemcitabine, cyclophosphamide, vincristine, prednisone), DHAP (dexamethasone, cisplatin, cytarabine), and ESHAP (etoposide, methylprednisolone, cytarabine, cisplatin), as well as pralatrexate or romidepsin. In 80% of patients achieving complete response and 30% achieving partial response to re-induction

chemotherapy, allogeneic or autologous SCT was included as consolidation therapy (based on expert opinion). The estimated annual cost of current treatment for incident and prevalent cases of rel/ref sALCL in the UK is approximately £2 million (£17,000 per patient). The main cost components are transplant (44%), drugs and administration (22%), AEs (17%), and long-term care (up to 2 years follow-up; 18%). **Conclusions.** sALCL is a rare disease with no standard of care in the rel/ref setting. Evidence from the systematic review showed that conventional chemotherapy approaches are not curative and are associated with poor outcomes. Autologous and allogeneic SCT may provide long-term benefit in patients with chemosensitive disease, but their utility is limited due to the inability of standard salvage treatments to deliver high complete response rates. Therefore, new efficacious therapies are needed. The budget impact of current treatments is relatively small due to low patient numbers.

0258

NON-HODGKIN LYMPHOMA IN PREGNANCY TENDS TO INVOLVE THE REPRODUCTIVE ORGANS: MYTH OR REALITY?

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Background. Hematological malignancies during gestation present a complex clinical scenario for mother and fetus. Data on pregnancy-associated non-Hodgkin lymphoma (PANHL) are scarce, limited to case reports and small retrospective series. Furthermore, PANHL pathological subtypes and organ involvement have not been fully characterized. **Aims.** The current study reviewed all trials and case reports published between 1967-2011, aiming to determine epidemiology, clinical presentation, management and outcome of PANHL. **Methods.** A systematic search for articles published since 1967 in PubMed, Medline and Google scholar databases regarding PANHL was performed. The words 'cancer', 'lymphoma', 'pregnancy' and 'gestation' were used as discriminator terms. Data on mother's age, gestational stage, histological lymphoma type, organ involvement, timing of therapy applied and mother and fetal outcomes were recorded and analyzed. Cases where lymphoma was diagnosed prior to pregnancy and those defined as Hodgkin or lymphoblastic lymphoma, were excluded from the analysis. **Results.** One hundred twenty two cases, reported in 85 papers, were analyzed. Patient's median age at diagnosis was 28 years, ranging between 15-42 years. Fourteen women (11.5%) were diagnosed during the 1st trimester of pregnancy and 41 (34%) in 2nd and 3rd trimesters each. Trimester of pregnancy was unmentioned for 26 cases. Indolent lymphomas accounted for 4.1% (n=5), aggressive NHLs for 42.6% (n=52; 39 -DLBCL, 13 - T cell lymphomas) and highly aggressive NHLs (HA-NHLs) for 43.4 % (n=53). The latter included 39 cases of Burkitt lymphoma (BL), 19 of them endemic, 9 unclassified HA-NHLs and 5 cases of immunoblastic lymphoma. Histological subtype was undetermined for 12 cases. Reproductive organ involvement (ROI), reported in 44.3% of patients (54/122), was most predominant in patients with BL. Sixty nine percent (n=27) of pregnancy-related BL presented with breast involvement: 25 with bilateral and 2 cases with unilateral masses. Additionally, 12 BL patients had ovarian involvement (5 bilateral and 7 unilateral) and 4 had uterus involvement, accounting for ROI (including breast, ovary and uterus) in about 84.6% (n=33) of BL patients. Notably, ROI was significantly higher in endemic BL (100%) vs non-endemic BL (70%) (p=0.02). The incidence of ROI in women diagnosed with PANHL other than BL was 25.3% (n=21, including T cell - 46.2%, immunoblastic - 40%, DLBCL - 23.1%, low-grade lymphoma - 40%, unclassifiable high-grade lymphoma - 22.22%); however, this percentage is still higher than reported in non-pregnant woman. Chemotherapy was administered during gestation in 54 cases (42.2%): 9 during the first trimester, 26 during the 2nd and 19 in 3rd trimester. A delayed (postpartum) treatment strategy was applied in 54 patients and 8 patients were not treated. Ninety nine pregnancies ended successfully (81.15%). Sixteen pregnancies were terminated early [spontaneously (n=5) or electively (n=11)] and 5 pregnancies ended in intrauterine fetal death. Within a median follow-up of 6 months from diagnosis, 68 patients (55.7%) survived and 53(43.44%) died, 31 in the HA-NHLs (58.5%). **Conclusions.** PANHL represents a unique entity, with frequent involvement of reproductive organs, mainly in BL. Collaborative prospective studies are required to characterize the epidemiology, delineate the mechanisms and evaluate the optimal management of these patients.

Myeloma - Biology 1

0259

WITHDRAWN

0260

HISTONE DEACETYLASE INHIBITOR INDUCED ANTI-MYELOMA ACTIVITY IS MEDIATED PRINCIPALLY VIA CLASS I DEACETYLASE INHIBITION

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Background. Histone deacetylases (HDACs) are a highly conserved group of enzymes that regulate a myriad of cellular functions through deacetylation of histones, transcription factors and molecular chaperones. Altered HDAC expression is significantly associated with a poor prognosis in a variety of malignancies, however, the expression pattern of HDAC and any potential correlation with patient outcome has not been characterized in multiple myeloma (MM). Furthermore, while HDACs inhibitors (HDACi) undergo continued therapeutic evaluation in MM, it is still not clear which HDAC(s) represent the optimal inhibitory target to induce maximal MM cell death. **Aims.** (i) Determine and compare HDAC expression in purified MM and normal plasma cells (PC) and correlate MM PC HDAC expression with overall survival (OS - diagnosis to death) and progression-free survival (PFS - diagnosis to first relapse/progression/death).(ii) Identify the HDAC that needs be inhibited to maximise HDACi-induced MM cell death. **Methods.** Quantitative RT-PCR was performed for *HDAC1-11* in normal (n=10) and MM PC (n=55) purified from patient bone marrow aspirates following appropriate consent. The OS and PFS of MM patients with high gene expression levels (>75th centile) compared to low levels (span75th centile) were derived utilising Kaplan-Meier survival plots. To determine the most suited HDACi for MM therapy, genetically heterogeneous myeloma cell lines (HMCL) and bone marrow mononuclear (BMMN) cells from MM patients were treated with a diverse range of HDACi for 48 hours (Figure 1).

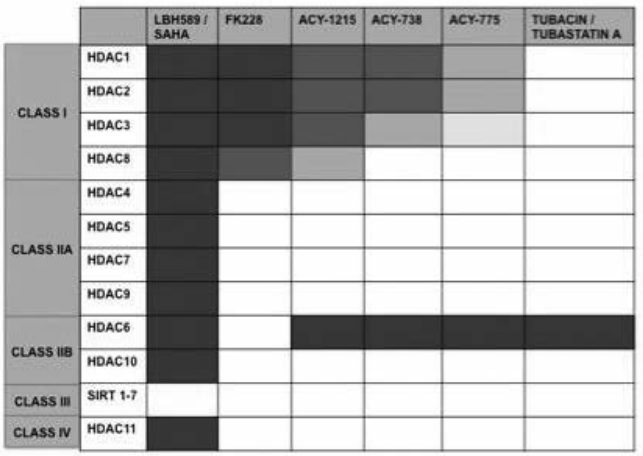

Figure 1. Image depicting inhibitory activity of HDACi against recombinant HDAC isoforms.Dark to lighter shades indicates strong to relatively weaker inhibition.

HDACi induced cell death of HMCL and primary MM cells was evaluated through flow cytometric enumeration of propidium iodide positive (PI+) or CD38hi/CD45neg/APO2.7pos cell populations, respectively. **Results.** *HDAC1, HDAC2 and HDAC6 over expression correlates with poor patient outcome*- Analyses of gene expression levels of *HDAC* in MM cells and normal PC revealed that median levels of all HDACs, with the exception of *HDAC1* and *HDAC11*, were significantly elevated in MM PC when compared to normal PC and that patients with the highest *HDAC1, 2* and *6* levels had a significantly shorter PFS from diagnosis.*Class I inhibition is critical to induce maximal MM*

*cell death*Six HMCLs (U266, OPM2, LP-1, RPMI8226, ANBL6 and XG-1) were treated with LBH589, FK228, ACY-1215, ACY-738 and ACY-775 and Tubastatin A for 48 hours. Measurement of PI+ cells at 48 hours indicated that in 6/6 cell lines tested, ACY-1215 and ACY-738 (both 10μM) caused cell death comparable to LBH589 and FK228 (both 50nM). ACY-775 and Tubastatin A induced comparable cell death in only 1/6 cell lines tested. Treatment of BMMN cells (n=10) with LBH589, SAHA, FK228 or tubacin also indicated that inhibiting Class I HDAC induced maximal MM cell death (75-90%) in 7/10 patient samples tested while inhibiting HDAC6 alone promoted killing comparable to the other HDACi inhibitors in only 5/10 patient samples tested. **Conclusions.** This study has established that altered *HDAC* levels correlate to poor outcome in MM patients and compounds with Class I HDAC inhibitory action induce maximal MM cell death. This fact not withstanding, specific HDAC6 inhibition demonstrates activity against some MM cells and this requires further evaluation.

0261

SERUM HEPCIDIN IS AN IMPORTANT MYELOMA VARIABLE AND AN INDEPENDENT PREDICTOR FOR SURVIVAL IN NEWLY DIAGNOSED MYELOMA PATIENTS

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Background. Hepcidin is a liver-derived hormone with a central role in iron regulationand in anemia of inflammation. Recently, it has been demonstrated that hepcidin is involved in the pathogenesis of anemia in Multiple Myeloma (MM). Furthermore, our group has recently shown in a small study of MM patients under treatment that, serum hepcidin may serve as a surrogate marker for MM and as a predictor for response to treatment. **Aims.** The aim of this study was to explore if serum hepcidin, measured at diagnosis, has a prognostic impact on overall survival (OS) and if it correlates with established adverse prognostic variables of MM in a large cohort of newly diagnosed patients. **Methods.** We studied 206 newly diagnosed, symptomatic MM patients: 105M/101F, median age 68 years (range: 33-91 years); 112 patients had IgG, 56 IgA, 32 light chain and 6 non-secretory MM. Sixty-four patients had ISS-1 MM, 69 ISS-2 and 73 ISS-3. Regarding frontline anti-myeloma treatment, 35 patients received MP, 28 patients receive bortezomib-based combinations (12 VD and 16 PAD), 90 received thalidomide-based regimens (32 CDT, 12 TVAD, 7 TD and 39 MPT), 23 received lenalidomide-based combinations (7 RD and 16 MPR), 11 patients received VDT and 19 patients received VAD. Sixty-three patients received autologous transplantation as a part of their initial MM treatment. All studied variables were measured before the administration of any kind of treatment, using fresh serum samples except for hepcidin and ferritin that were measured in frozen samples. Serum hepcidin was measured using a C-ELISA methodology, as previously described (Ganz et al. Blood 2008). Mann-Whitney U-test, One-Way ANOVA and Pearson's χ^2 test were used for correlations. Cox-regression was used to determine possible independent predictive factors for OS. *p* value <0.05 was considered as statistically significant. **Results.** The median serum hepcidin levels for all patients were 145ng/mL (range 0.01-2336ng/mL). Serum hepcidin positively correlated with ISS (p<0.001), serum creatinine (p<0.001; r=0.7) LDH (p=0.002; r=0.2), β2M (<0.001; r=0.6), C-reactive protein (p=0.03; r=0.2) ferritin (p=0.006; r=0.4) and transferrin saturation (p=0.002; r=0.4). Serum hepcidin also showed negative correlations with albumin (p<0.001; r=0.4), hemoglobin (p<0.001; r=0.5) and platelet counts (p=0.002; r=0.2). The univariate analysis showed that serum hepcidin, age, ISS, creatinine, LDH, β2M, albumin and hemoglobin were significant predictors for OS (p<0.05 for all parameters), whereas type of MM, C-reactive protein, platelet counts and free-light chain ratio did not predict for OS (p>0.05). The multivariate analysis showed that serum hepcidin, albumin and age, independently predicted for OS [(p<0.001) (95% CI:1.001-1.002),(p=0.001) (95%CI:0.34-0.75) and (p=0.003) (95%CI:1.011-1.057), respectively]. With a cut-off value of baseline serum hepcidin 286ng/mL, which represents the adult 95% upper normal limit- as previously described -and with a median follow-up of 47 months, the OS of patients with hepcidin >286ng/mL was 32 months (95% CI: 25-40) and of patients with normal serum hepcidin (≤286ng/mL) 62 months (95% CI: 39-84; p<0.001). **Conclusions.** Serum hepcidin strongly correlates with adverse clinical and biological myeloma features and is a strong predictor for survival in newly diagnosed symptomatic MM patients.

0262

DEREGULATION OF SIRTUINS PRESENTS IT AS NOVEL THERAPEUTIC TARGETS IN MYELOMA

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Background. The silent information regulator 2 (Sir2) family are highly conserved genes consisting of seven mammalian members (SIRT1-7). SIRTs orchestrate a variety of cellular functions including gene silencing, cell cycle and apoptosis, genomic stability and energy homeostatis through deacetylation and ADP ribosylation of proteins. Therefore, they are associated with numerous pathologies including metabolic diseases, neurodegenerative disorders and cancer. Evidence indicates that SIRT1-3 play contradictory roles in cancer and can function as a context-dependent tumor suppressor or oncogenic factor. This dual role renders them attractive therapeutic targets for anti-cancer therapy. In multiple myeloma (MM) the potential correlation of *SIRT* expression with patient outcome and the impact of modulating SIRT activity has not yet been determined. **Aims.**(i) Determine and compare *SIRT* expression levels in purified MM cells and normal plasma cells (PC) and correlate expression with overall survival (OS - diagnosis to death) and progression-free survival (PFS - diagnosis to first relapse/progression/death) of MM patients.(ii) Determine the impact of modulating SIRTs in genetically heterogeneous human myeloma cell lines (HMCL).**Methods.** Bone marrow aspirates from MM and normal patients were obtained following consent. Total RNA and cDNA were prepared from purified normal (n=10) and MM PC (n=55) and quantitative RT-PCR performed for *SIRT1-7*. The OS and PFS of MM patients with high gene expression levels (>75th centile) compared to low levels (<75th centile) were derived utilising Kaplan-Meier survival plots. To determine the impact of inhibiting SIRTs, HMCL were treated with commercially available SIRT inhibitors, EX-527 (inhibits SIRT1) and salermide (inhibits SIRT1 and SIRT2) for 48 hours and the proportion of cell death was determined through flow cytometric enumeration of propidium iodide positive (PI+) cells. **Results.** Gene expression analysis revealed that median levels of *SIRT1, 3, 5* and *7* were significantly elevated in MM PC compared to normal PC (p<0.01). MM patients exhibiting elevated *SIRT1* (1334 vs 2188 days; p=0.039)*, SIRT2* (844 vs 2272 days; p=0.003) and *SIRT5* (809 vs 2272 days; p=0.0004) displayed shorter OS compared to patients with lower *SIRT* levels, while patients with elevated *SIRT2* also displayed shorter PFS (649 vs 1050 days; p=0.038). Since *SIRT* gene expression levels were upregulated in MM PC from patients, the potential of inhibiting SIRTs in HMCL was explored. Six HMCLs (U266, OPM2, NCIH929, OCIMY1, ANBL6 and XG-1) were treated with EX527 and salermide (both at 25μM, 50μM and 100μM) for 48 hours. Measurement of PI+ cells at 48 hours indicated that EX527 induced cell killing in 6/6 cell lines tested at the highest dose (100 μM; 20-40%), while salermide was able to promote higher cytotoxicity at a lower dose (50μM; 50-75%), suggesting that inhibition of both SIRT1 and SIRT2 is superior to SIRT1 inhibition alone in inducing MM cell death. **Conclusions.** Augmented *SIRT* levels in MM patients correlate to poor outcome in patients signifying that SIRTs may represent novel predictors of adverse outcome in newly diagnosed MM patients. Furthermore, inhibiting SIRT1 and SIRT2 promotes MM cell death, providing a rationale for the exploration of SIRT inhibitors as anti-MM agents.

0263

HIF-1α INHIBITION PROMOTES CELL CYCLE ARREST IN MULTIPLE MYELOMA

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Background. Hypoxia-inducible factor-1 alpha (HIF-1α) plays a critical role in survival and angiogenesis and is associated with poor prognosis in solid tumors. The role of HIF-1α in hematological malignancies is not completely known. In particular in multiple myeloma (MM) HIF-1α is constitutively expressed in about 35% of patients, and HIF-1α knockdown cell lines have shown higher sensitivity to standard chemotherapy, suggesting HIF-1α inhibition as a possible therapeutic strategy. **Aims**. In the present study, we explored the effect of EZN2968, an locked nucleic acid antisense oligonucleotide against HIF-1α, as a molecu-

lar target in MM. **Methods.** A panel of MM cell lines (MM1S, U266, OPM2, RPMI8226) and primary samples from MM patients were cultured *in vitro* in the presence of EZN2968 (20 μM) in normoxia ($p0_2$ 21%) and hypoxia ($p0_2$ 1%) condition. **Results.** Baseline expression of HIF-1α was initially investigated on MM cell lines and primary samples from MM patients. Under normoxia culture condition, HIF-1α mRNA and protein expression was detectable in all MM cell lines tested and in $CD138^+$ cells from newly diagnosed MM patients samples. Significant up-regulation of HIF-1α protein expression was observed after a short incubation with IL6 or IGF-I, confirming that HIF-1α can be further induced by biological stimuli. EZN2968 efficiently induces a selective and stable down-modulation of HIF-1α mRNA and protein expression either in normoxia or hypoxia conditions, without affecting the expression of other family members of hypoxia inducible transcription factors (HIF2 α). Moreover, we tested whether vascular endothelial growth factor (VEGF), a key transcription factor downstream target of HIF-1α, was modulated by EZN2968. The secretion of VEGF released by MM cell lines cultured in the presence or absence of EZN2968 decreased in a time dependent manner in the treated compared to untreated samples. Treatment with EZN2968 (up to 72hrs) gave rise to a progressive accumulation of cells in the S-phase and a concomitant reduction of G2/M phase. In addition, the subG0 phase was moderately increased. The analysis of p21 and p27, two keys cyclin-dependent kinase inhibitors controlling cell cycle check point, showed upregulation of protein levels. These results suggest that HIF-1α inhibition is sufficient for cell cycle arrest in normoxia. We next evaluated cell death induced by HIF-1α inhibition. AnnexinV/PI staining showed that incubation with EZN2968, moderately increased the percentage of Annexin V positive cells compared to untreated cells (30% vs 8%, respectively after 72h of treatment). In a time course experiment, we observed cleavage of PARP after 48 hours of treatment with EZN2968, suggesting the activation of an apoptotic pathway in response to HIF-1α depletion. **Summary.** In this study we provide evidence that HIF-1α, even in the absence of hypoxia signal, is expressed in MM plasma cells and is further inducible by bone marrow milieu stimuli, and that its inhibition is sufficient to induce a permanent cell cycle arrest. Our data support the idea that HIF-1α inhibition may suppress tumor growth by preventing proliferation of plasma cells through p21 activation.

0264

A FISH STUDY TO CONFIRM THAT ISOLATED 7Q DELETIONS AND ISOLATED MONOSOMIES 7 SHOULD BE NO MORE INCLUDED WITHIN THE SAME PROGNOSTIC CATEGORY

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Background. In MDS isolateddel(7q) and -7 are included in different categories by the "New Comprehensive Cytogenetic Scoring System". **Aims.** The present study was aimed at evaluating whether a series of BAC and commercial probes exploring bands 7q22, 7q31, 7q34-q35-q36 were able to reveal cryptic chromosome 7 lesions in 79 chromosomally normal patients and in 24 del(7q) patients. An additional goal was to assess whetherdel(7q) and -7 determined a significant different overall survival and a progression-free interval. **Methods.** The 121 patients (79 with a normal karyotype, 24 with an isolated 7q- and 18 with an isolated -7) analysed by the present study were included in a series of 637 patients who came to our observation in the period January 2000-December 2010. FISH probes obtained from Kreatech (Amsterdam, NL), Abbot Molecular Inc. (Chicago, Il,USA),BACPACResourcesCenter at C.H.O.R.I. (Oakland,USA). FISH was carried out as previously described. The following probes were used: ON MDS 7q- (7q22/7q35), RP11-51M22 (7q31), RP11-122A11 (7q34), RP11-992C19. I-FISH cut-off values, obtained after analysing 300 nuclei from ten normal samples, were fixed at 10%. **Results.** CC revealed a -7 and adel(7q) in 18 and 24 patients respectively. In 7 of these last patients (29.2%) FISH did not confirmdel(7q). Noteworthy, among the 79 chromosomally normal patients 8 (10.1%) presented an abnormal FISH pattern: 2 patients (one RCMD, one RAEB-1) showed a -7, 5 patients (one RA, 3 RCMD, one RAEB-1) adel(7q) and one RA patient an amplification of band 7q35. The chromosomal bands most commonly involved in deletions were 7q22 (15 patients) and 7q22 (7 patients). Interestingly, a RA patient with adel(7)(q22) at clinical diagnosis showed a progressive increase in the extension of the deleted area and eventually developed a -7. Thus, isolated chromosome 7 abnormalities were revealed in a total of 43/121 patients (35.5%): 20 (16.5%) presented a -7 and 22 (18.2%) adel(7q). There were 15 females and 27 males, median age 63 years (range 28-89). Median follow-up was 10 months (range 1-103). According to WHO classification, 4 patients were classified as RA, 14 as RCMD, 12 as RAEB-1 and 13 as RAEB-2. According to IPSS 2 patients were considered low-risk, 8 intermediate-1 risk, 20 int-2 risk and 13 high-risk.Del(7q) was associated with higher platelets counts, early MDS (11/22 versus 7/20) and lower IPSS risk subtypes (6/22 versus 3/20). Death occurred in 9 (41%) 7q- patients and in 10 (50%) -7 patients (p=0.01), whereas clinical evolution occurred in 12 (54.5%) and 14 (70%) patients respectively (p=0.01). Considering the 8 patients with a cryptic chromosome 7 defect on FISH studies, one -7 patient died and 4 patients, two each with a -7 and adel(7q), progressed to AML. The RA patient with a 7q35 amplification presented a stable disease. **Conclusions.** i) FISH is an effective method to reveal cryptic chromosome 7 defects and should be routinely applied with CC; ii) 7q deletions and -7 should be included within different prognostic categories as proposed by the NCCSS.

0265

MINIMAL RESIDUAL DISEASE BY MULTIPARAMETRIC FLOW CYTOMETRY IS A USEFUL APPROACH TO IDENTIFY PATIENTS AT DIFFERENT RISK OF PROGRESSION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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Background. High dose therapy with autologous stem cell support is considered standard for multiple myeloma (MM) patients eligible for the procedure. Most of the patients, however, will eventually relapse. Maintenance therapy is an option for sustaining response but at certain hazards. It is therefore important to define biomarkers of potential aid for the identification of patients at high risk of progression that might benefit additional treatment. **Aims.** To estimate the amount of residual myelomatous plasma cells by MFC in the bone marrow of MM patients after autologous stem cell transplantation (ASCT) and the impact on the course of the disease. **Methods.** Minimal residual disease (MRD) was assessed by 8-color MFC on day +100 in bone marrow aspirates of 39 MM patients who underwent ASCT using panels of monoclonal antibodies designed on the basis of the individual phenotypes at diagnosis, including CD138, CD38 and CD45 for gating purposes, and CD19, CD20, CD27, CD28, CD56, and CD117 to detect plasma cells (PC) [FACSCanto II (BD), Infinicyt software (Cytognos)]. In addition, the proportion of abnormal PC (APC) out of total PC in the sample was determined. Response assessment was performed at day+100 and at the end of the study after a mean of 12 months of follow up (range 4-32) applying the IMWG 2006 criteria. Time to progression (TTP) was calculated from the day of the autograft infusion to the day of relapse or progression. The impact of major demographic, clinical, laboratory and therapeutic variables was assessed by Kaplan-Meier and log-rank test. **Results.** FCM showed detectable abnormal PCs in all patients enrolled in the study. The median MRD level was 0.03% (range 0.002%-8.55%) from all cells acquired. These comprised 1.88% to 99.4% (median 22.9%) within the total PC compartment. At latest follow-up, 12 (30%) patients suffered disease progression and 27(70%) patients sustained their response. Data analysis showed that only ISS stage (log rank, p=0.042) and complete response (CR) at day+100 (Pearson chi-square, p<0.000) had statistically significant impact on TTP and persistent remission at final follow up respectively, while age, gender, initial phenotype, chemotherapy regimens prior to transplantation had no prognostic influence. On the other hand, MRD levels significantly influenced TTP. None of the 18 patients with MRD <0.02%, even those who failed to achieve CR at day+100, relapsed at the end of the study, while 12/21 patients with higher MRD levels, irrespective of CR at day+100, suffered disease progression after a median TTP of 13 months (0% vs. 57%, log rank p=0.0016). In addition, the proportion of ACP of all PCs also emerged as an important predictive marker for unsustained therapy response as 5% value proved to be the most discriminative regarding TTP (log rank p=0.036). **Conclusions.** Our results show that MRD evaluation by MFC is a very useful approach to identify patients at different risk of progression. Higher MRD levels are predictive of inferior TTP. MFC may contribute to evidence based therapeutic decisions for "patient-specific maintenance therapy". **Acknowledgements.** The study was supported by National Science Fund, Ministry of Education, Youth and Science.

0266

CXCR4 PROMOTES MALIGNANT PHENOTYPE AND TUMORIGENESIS OF MULTIPLE MYELOMA AND NON HODGKIN LYMPHOMA IN VITRO AND *in vivo* IN ANIMAL MODEL

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Background. CXCR4/CXCL12 axis regulates a diverse array of cellular processes, including leukocyte trafficking, HSCs homing and B-cell lymphopoiesis. CXCR4 has been also implicated in the progression of hematological malignancies. CXCR4 is highly expressed by a variety of B cell neoplasms, such as CLL, MM and NHL. CXCR4 is critical regulator of MM homing. In addition, CXCR4 mediates migration, survival and chemoresistance of CLL cells. However, further investigation is required to reveal the role of CXCR4 in tumor-initiating cell phenotype and unravel the specific molecular mechanisms regulated by the CXCR4/CXCL12 axis in B cell malignancies. **Results.** Surface CXCR4 is commonly down-regulated in MM cell lines. To study the functional role of CXCR4 in MM we stably over-expressed CXCR4 in MM cells ARH77 and RPMI8226. As complementary research strategy, we blocked endogenous CXCR4 in Burkitt lymphoma cell line BL-2, that highly express surface CXCR4, using anti-CXCR4 shRNA construct. Enhanced CXCR4 expression in MM cell lines significantly increased their *in vitro* survival in serum-deprivation conditions ($p<0.01$). In addition, we found that elevated CXCR4 increased chemoresistance of MM cells to melphalan and bortezomib. Also, up-regulation of CXCR4 promoted MM soft-agar colony formation, significantly increasing colonies number and size. This reflects increased tumorigenic capacity of CXCR4-expressing cells. Respectively, CXCR4 blockade with shRNA inhibited the survival of lymphoma cells *in vitro*. Elevated CXCR4 prominently increased CXCL12-dependent transwell migration of MM cells ($p<0.01$), as well as their adhesion to fibronectin, laminin and BMSCs. In accordance, adhesion of BL-2 cells with silenced CXCR4 was significantly abrogated ($p<0.01$). Moreover, we found that enhanced CXCR4 significantly elevated cell-surface integrin VLA-4 on RPMI8226 cells. Moreover, we observed direct correlation between CXCR4 and VLA-4 expression levels on primary malignant cells in BM samples from MM and NHL patients. Next, we found that elevated CXCR4 increased the basic levels of pERK1/2 and pAKT in MM cells, and promoted their prolonged activation in response to CXCL12. Accordingly, increased resistance of CXCR4-expressing cells to ERK and PI3K inhibitors was observed. Importantly, JNK and Jak/STAT inhibitors effectively blocked RPMI8226-CXCR4 cell growth, identifying JNK and Jak/STAT pathways as potential therapeutic targets in CXCR4-promoted tumorigenesis. Lastly, we determined the role of CXCR4 in tumor development *in vivo*. CXCR4-expressing MM cells and CXCR4-silenced BL-2 cells, as well as corresponding parental cell lines, were subcutaneously injected into NOD/SCID mice. CXCR4-expressing, but not parental, MM cells produced detectable rapid-growing tumors already 10 days after the injection. Oppositely, CXCR4 silencing in BL-2 cells significantly ($p<0.001$) inhibited local tumor growth and fully prevented NHL spread to BM. These findings indicate that CXCR4 provides aggressive phenotype of MM and NHL *in vivo*. **Conclusions.** Taken together, our findings clearly demonstrate the important pathophysiologic role of CXCR4 in B cell malignancies. Furthermore, for the first time, we provide the evidence for CXCR4 oncogenic potential in MM, showing that CXCR4 promotes the clonogenic growth of MM cells. Our model may further serve to elucidate CXCR4-regulated molecular events involved in the pathogenesis of MM and NHL, and strongly support targeting CXCR4 as therapeutic tool in B cell neoplasms.

0267

EX VIVO PHARMACOLOGICAL EVALUATION OF BORTEZOMIB IN MULTIPLE MYELOMA (MM) PATIENT SAMPLES

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Background. Bortezomib has become established as a standard treatment for MM, both in relapsed patients and the newly diagnosed, but a proportion of patients are resistant to the drug. To further explore the *ex vivo* pharmacology of this drug, bone marrow samples from 150 patients diagnosed with MM were evaluated in a cell-based assay to determine the ability of bortezomib to induce cell death in the malignant population. The *ex vivo* efficacy and potency of bortezomib was determined, alone and in drug combinations that represent current treatment protocols. **Aims.** This study sought to identify patient samples that were resistant to bortezomib in an *ex vivo* test and to determine possible alternative treatments by evaluating the *ex vivo* pharmacology of the drug. **Methods.** Working with 16 hospitals across Spain, bone-marrow samples from patients diagnosed with MM were obtained, having first received informed consent, and processed within 24 hours of extraction. The sample was diluted in its entirety (retaining erythrocytes and plasma) and plated into 96-well assay plates containing the pharmacological agents. The plates were incubated for 48-hours, and then prepared for analysis by our flow cytometry-based ExviTech© platform. The percentage of plasma cell death was determined via labeling with appropriate monoclonal antibodies and AnnexinV-FITC. Dose/response curves of bortezomib were run to establish the efficacy and potency for each patient. Dexamethasone, melphalan, prednisolone, cyclophosphamide and doxorubicin were also tested, alone and in combination with bortezomib, as a reference to current treatment protocols.

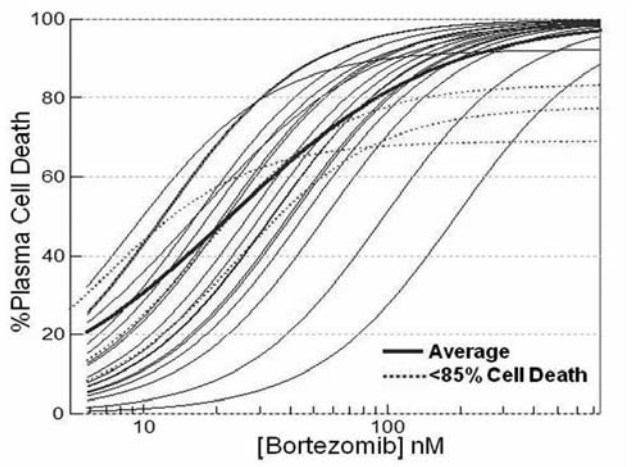

Figure 1. Twenty-two representative *ex vivo* dose/response curves to Bortezomib.

Results. Bortezomib eliminated over 90% of malignant cells in 97% of samples at high doses (Figure 1). In three samples a population of 15-30% of malignant cells was not killed with bortezomib (dashed lines), even at high concentrations, thus identifying the emergence of a potential bortezomib-resistant clone. There was a very wide spread in the bortezomib potency across the patient population, the EC50 ranging from 1 to 200nM (average of 30nM). The other drugs tested varied even more in efficacy (from <20% to >90% cell death) and potencies ranged over more than three orders of magnitude, displaying wide variations from sample to sample. For all patients tested, at least one drug, or one

drug-combination reached an efficacy of >85% cell death, suggesting a potentially efficacious treatment. **Conclusions.** The results of this study displayed a wide variability in the *ex vivo* activity of drugs, which corresponds to the wide a range of responses that are seen in the clinical outcome of the patients. By testing the drugs used in the treatment protocols for MM directly on patient samples, a pharmacological based model could be developed to infer drug resistance or sensitivity, patient by patient. This is similar to the Individualized Tumor Response Testing that has been documented over the last 20 years, albeit using a flow cytometry based assay and incorporating drug combinations evaluated in whole blood. The ultimate goal would be the development of a personalized medicine test for MM. This test would also provide an opportunity to explore reported synergy between bortezomib and other drugs such as doxorubicin and melphalan.

0268

GENETIC, EPIGENETIC AND DNA DAMAGE RESPONSE ALTERATIONS AS MOLECULAR PREDICTORS OF RESPONSE TO MULTIPLE MYELOMA THERAPY

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Background. Multiple myeloma (MM) is a plasma-cell malignancy in which several genetic and epigenetic changes that are implicated in the biology of the disease. **Aims.** To evaluate epigenetic modifications and alterations in cellular DNA damage response pathways in myeloma patients who underwent high dose chemotherapy and autologous transplantation (ASCT). **Methods.** We studied 12 healthy volunteers (7M/5F; median age 41 years) and 70 MM patients (25M/45F; median age 53 years) who underwent high-dose melphalan therapy with ASCT. All patients provided informed consent according to institutional guidelines. Forty-eight patients achieved an objective response to ACST (responders) and twenty-two patients did not (non-responders). Peripheral blood mononuclear cells (PBMCs) were isolated prior to chemotherapy andwere *ex vivo*treated with melphalan. Three molecular end-points (chromatin condensation, transcription activity, DNA repair in both transcribed and non-transcribed DNA strands) were measured in four genomic loci (beta-actin, p53, N-ras and delta-globin genes). Furthermore, accumulation of p53 protein, inhibition and recovery of total RNA synthesis, as well as induction of apoptosis were also studied. **Results.** In all subjects, beta-actin, p53 and N-ras genes were transcriptionally active. Importantly, delta-globin gene was silent in all healthy volunteers, while an induction of the transcription activity of this gene was found in 49/70 (70%) of MM patients.In all subjects, 5'- to 3'-end gradients of chromatin condensation and repair efficiency were observed along the transcribed strand of the active genes, with higher looseness of chromatin structure and faster repair at the 5'-end. Nosignificant difference in the repair efficiency was found between MM patients in regionsoutside the genes as well as in the non-transcribed strand of active genes. Notably, responders showed a much slower repair efficiency of the transcribed strand than non-responders, with the difference being greater and statistically significant at the 5'-end ($p<0.001$).PBMCs from all healthy volunteers showed evidence of p53 protein accumulation at doses as low as 10 µg/mL melphalan, responders at 75 µg/mL, while non-responders required doses of at least 100 µg/mL. These results suggest that cells with lower repair activity (PBMCs from responders) are more sensitive to melphalan-induced p53 accumulation than PBMCs from non-responders having higher levels of preferential repair.PBMCs from non-responders had almost fully recovered total RNA synthesis to control levels by 6h following exposure to 100µg/ml melphalan. In contrast, PBMCs from all healthy volunteers and most responders (39/48) showed a more extensive and prolonged inhibition of RNA synthesis, suggesting that, the ability to preferentially repair the transcribed strand of the active genes correlates with rapid recovery of total RNA synthesis.Finally, induction of apoptosis was evident following 10µg/ml melphalan in all healthy volunteers, 75µg/ml in most responders (35/48) and at least 100 µg/mL in non-responders, suggesting that PBMCs from responders correlated with an increased susceptibility to melphalan-induced apoptosis. **Summary and Conclusions.** This study provides new data on the prediction value of specific genetic alterations, epigenetic changes and key components of DNA damage response pathways for response to high dose melphalan and ASCT in myeloma patients. These results can be translated in novel biomarkers for prediction of response to MM therapy.

0269

EVIDENCES OF EARLY SENESCENCE IN MULTIPLE MYELOMA BONE MARROW MESENCHYMAL STROMAL CELLS (MM BM-MSCS)

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Background. It is now well established that the bone marrow (BM) constitutes a microenvironment required for differentiation, maintenance, expansion, and development of drug resistance of the multiple myeloma (MM) cell clone. Previous studies have suggested that direct and indirect interactions between MM plasma cells (MM-PCs) and BM-MSCs result in constitutive abnormalities in BM-MSCs. **Aims.** The aims of our study were to further investigate the constitutive differences of MM BM-MSCs compared to healthy donors (HD) BM-MSCs and to evaluate the impact of actual treatments on MM BM-MSCs. **Methods.** We carried out microarray analysis on BM-MSCs derived from MM patients (Untreated or previously treated with Thalidomide, Lenalidomide and Bortezomib) and healthy donors with an Affymetrix Genechip Human Genome U133 Plus 2.0 Array. In addition, we evaluated diverse characteristics of MM-MSCs such as proliferation capacity (CFU-F, doubling time), osteoblastogenesis (calcium deposits, ALP activity), cytokine and chemokine expression profile (ELISA), hematopoietic support (Bl-CFL, LTC-IC) and immunomodulatory activity (MLR). **Results.** We demonstrated that MM BM-MSCs, compared to HD BM-MSCs, presented an early senescent profile with expression of senescence-associated β-galactosidase (68.10 vs 20.23% of positive cells; $p=0.0002$; $n=11$), increased cell size (32.55 vs 31.32 µm; $p<0.0001$; $n=10$), reduced proliferation capacity (7156 vs 200298 CFU-F at passage 1; $p=0.0173$; $n=6$) and characteristic expression of members of the senescence associated secretion profile (SASP). We observed alterations in the osteoblastogenesis with a reduction of calcium deposits (8.74 vs 14.81 mg/µg of protein; $p=0.0023$; $n=7$) and an early rise of ALP activity (0.037 vs 0.018 U/µg of protein; $p=0.0126$; $n=9$). Finally, the hematopoietic support and immunomodulatory activity of MM BM-MSCs were also altered. For all these experiments, the actual MM treatments were able to partially reduce the MM BM-MSCs abnormalities (secreted factors, proliferation). **Conclusions.** We hypothesize that MM-PCs and MM BM-MSCs are co-dependent since separated *in vitro* cultures induce apoptosis or senescence and co-cultures permit the survival of both. So, it is of great importance to evaluate the impact of the MM-PCs eradication (complete and partial remissions) and of the treatments responsible of this eradication on MM BM-MSCs. Indeed, the disturbance of the bone marrow environment by senescent MM BM-MSCs would be sufficient to induce relapse or secondary cancers such as myelodysplasia and leukemia.

0270

HYPOMETHYLATION OF ENDOGENOUS RETROTRANSPOSONS IS CORRELATED WITH FREQUENT CHROMOSOMAL DELETIONS AND POOR PROGNOSIS IN MULTIPLE MYELOMA

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Background. Aberrant genome-wide hypomethylation is thought to be related to tumorigenesis through its promotion of genomic instability. Since the methylation of endogenous retrotransposons, which make up at least 42% of the human genome, including long interspersed nuclear element 1 (LINE-1) and short interspersed element Alu, plays an important role in the silencing of transposon-mediated mutagenesis, hypomethylation of retrotransposons in human tumors may lead to their reactivation. However, the role of the methylation of retrotransposons in multiple myeloma (MM), characterized by various chromosomal aberrations, remains to be elucidated. **Aims.** In this study, we investigated the methylation levels of retrotransposons, their correlation with the chromosomal aberrations, and their clinical relevance in predicting prognosis. **Methods.** We analyzed the methylation levels of LINE-1 and Alu as well as DNA copy number alterations in purified bone marrow plasma cells from patients with MM (n=74), patients with monoclonal gammopathy of undetermined significance (MGUS, n=7), and normal plasma cell providers (NPC, n=4) using bisulfite-pyrosequencing and array-based comparative genomic hybridization (aCGH), respectively. **Results.** We found a progressive and significant methylation decrease in Alu and LINE-1 from normal controls to MGUSs and MMs. Secondarily, the numbers of deleted probes of aCGH were significantly inversely correlated with the methylation levels of LINE-1 as well as of Alu. Important-

ly, hypomethylation of LINE-1 was well associated with frequent chromosomal deletions, including the deletion of chromosome 13. Finally, we observed significantly poorer prognosis in the lower LINE-1 methylation group compared with the higher group. **Summary and Conclusions.** Our data suggest that the hypomethylation of retrotransposons, especially LINE-1, correlates significantly with malignant levels, the frequency of chromosomal deletions, and prognosis in MM, and that LINE-1 methylation could be a useful marker for risk stratification.

0271

RECONSTRUCTING THE HUMAN HEMATOPOIETIC NICHE: OPPORTUNITIES FOR STUDYING NORMAL AND MALIGNANT HEMATOPOIESIS

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Background. Interactions with the hematopoietic niche in the bone marrow (BM) microenvironment are essential for hematopoietic stem cell (HSC) self-renewal. In addition, in hematological malignancies this niche is considered to serve as a sanctuary site for leukemic stem cells during chemotherapy, and to contribute to disease relapse. Although many advances have been made in understanding how the niche regulates HSC self-renewal and confers therapy resistance, most of this knowledge is based on genetic loss- or gain-of-function murine models. **Aims.** Since current models do not recapitulate the human physiology, there is a need for models that more closely resemble the human niche. **Methods.** We developed a unique humanized model, by implementing a novel scaffold-based technology for generating a human bone environment in RAG2$^{-/-}$gc$^{-/-}$-mice that facilitated engraftment of normal and malignant hematopoietic cells **Results.** Inoculation of mice carrying a humanized environment, with normal human CD34^{+} hematopoietic progenitor cells, isolated from umbilical cord blood, resulted in homing to the human bone environment and the generation of human hematopoietic cells of distinct lineages, but more importantly also the engraftment of CD34^{+} cells themselves. In a next series of experiments the supportive nature of the humanized niche was further investigated with patient-derived acute myeloid leukemia (pAML) and multiple myeloma (pMM) cells, two hematopoietic malignancies that are highly dependent on the BM microenvironment for survival and growth. Inoculation of the humanized mice with pAML cells, obtained from a poor-risk patient (M1; complex karyotype) or cells from a good risk AML patient (M4; inv(16)) revealed the ability of the reconstructed human bone environment to support outgrowth of the leukemia with the cells having a similar phenotype as those from the patient sample. Interestingly, engraftment of good risk AML samples, including inv(16), has been reported to be very difficult in the NOD/SCID-based AML xenotransplant model. Retransplantation of leukemic cells harvested from the scaffolds after passage 1, again engrafted in secondary scaffolds and showed after passage 2, identical outgrowth as in passage 1. The humanized model that we developed was further substantiated by the ability to support the outgrowth of pMM from 7 out of 7 patients. MM is a hematological malignancy that fails to grow in mouse tissues without extra support, *e.g*. fetal human bone chips. Moreover, the outgrowth of pMM in our humanized model is accompanied by an increase in osteoclast activity, indicating the presence of bone resorption, one of the most relevant clinical sequelae of MM.In addition, by gene-marking pMM cells with luciferase and using bioluminescent imaging, we were able to follow myeloma outgrowth in time. Treatment of pMM-bearing mice with identical drugs as given to the patients showed that the pMM cells growing in the humanized environment in the mice responded similar as the MM patients. **Conclusions.** This novel human-mouse hybrid model creates unprecedented opportunities to investigate species-specific microenvironmental influences on normal and malignant hematopoietic development, and to develop and personalize cancer treatment strategies

0272

THE USE OF CD138 POSITIVELY SELECTED MARROW SAMPLES INCREASES THE APPLICABILITY OF MINIMAL RESIDUAL DISEASE ASSESSMENT BY PCR IN PATIENTS WITH MULTIPLE MYELOMA

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Background. In patients with multiple myeloma, the use of ASCT and novel agents is associated with high rates of complete response (CR) and thus, new methods assessing higher degrees of disease eradication are required. Minimal residual disease (MRD) detection by immunoglobulin heavy-chain real-time quantitative PCR (IGH RQ-PCR) and multiparameter flow cytometry (MFC) has proved to be of prognostic value in MM patients. Compared with MFC, RQ-PCR seems to have higher sensitivity but lower applicability due to inadequate diagnostic samples and/or lack of a suitable molecular marker. New strategies aimed at improving the applicability and performance of the PCR are thus needed. **Aims.** We have evaluated the use of CD138 positively selected bone marrow samples to identify a molecular target for MRD assessment by PCR in patients with MM. A fraction of each sample was used for CD138+ selection and the rest served as a reference control. **Methods.** Cell samples were obtained from bone marrow aspirates of 25 patients included in GEM/PETHEMA protocols. CD138+ plasma cell isolation was carried out using the AutoMACs separation system. High molecular weight DNA was isolated using DNAzol reagent. All samples were tested for amplification of complete (VDJ), incomplete (DJ) and Kde (Vk-Kde/intronRSS-Kde) rearrangements according to the BIOMED-2 Concerted Action. Germline IGH genes from complete VDJ and partial DJ rearrangements were identified using public IMGT/V-QUEST and BLAST databases, respectively. Kde and intron-RSS segments were identified by direct comparison with their corresponding germline sequences and Vk segments by using the IMGT database. **Results.** We first assessed clonality in all the 25 samples by amplifying VDJ, DJ and Kde rearrangements. Within the CD138+ selected group, VDJ rearrangements were detected in all cases (100%), DJ in 16 (64%) and Kde in 18 (72%) cases, whereas in the control samples VDJ, DJ and Kde rearrangements were detected in 19 (76%), 11 (44%) and 12 (48%) cases, respectively. As a result, all samples within the CD138+ selected group were suitable for sequencing, whereas only 21 (84%) of the reference samples were so. VDJ sequencing was successful in 22 samples (88%) within the CD138+ selected group and in 12 of the 19 (63%) unselected samples. By contrast, only minor differences in sequence efficacy were observed for DJ (seven and five cases were successfully sequenced among the CD138+ and unfractionated samples, respectively) and Kde (11 and nine cases in each group). In summary, 96% (24/25) of cases within the CD138+ selected group were suitable for PCR-based MRD assessment, 14 (56%) of them having two or more potential specific markers while in the unselected samples only 60% (15/25) of cases were suitable for MRD studies, with eight (32%) of them having two or more markers available. **Summary and Conclusions.** We conclude that the use of CD138 positively selected bone marrow samples increases the applicability of MRD studies by PCR in patients with MM (in our study, from 60% in unselected samples to 96% in CD138+ selected samples) and propose that it should be considered for inclusion as part of routine practice.noepuig@gmail.com

0273

THE ROLE OF TUMOR PROMOTING IMMUNE SUPPRESSIVE MYELOID CELLS IN THE MULTIPLE MYELOMA BONE MARROW MICROENVIRONMENT

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Background. The interaction of myeloma (MM) cells with bone marrow accessory cells and/or the extracellular matrix induces genomic, epigenomic and functional changes which promote tumor development, progression, cell adhesion mediated-drug resistance (CAM-DR), and immune suppression. To develop the most efficient anti-MM treatment strategy and prevent tumor escape from immune recognition, both enhancing anti-MM effector immune response and overcoming MM-induced immune suppression is essential. Suppressive immune cells including myeloid derived suppressor cells (MDSC), regulatory T cells (Treg) and IL-17 secreting Th (Th17) cells act as tumor promoters and suppressors of effector immune response, and therefore represent a significant barrier to current anti-tumor therapeutic strategies. **Aims.** Since we and others have reported increased numbers of Treg and Th17 cells in MM, we here assessed tumor promoting, immune suppressive role of MDSCs in both peripheral blood (PB) and bone marrow (BM) of patients with MM compared to healthy donors. **Methods.** MDSCs were phenotypically characterized using flow cytometry analysis. MDSCs and autologous T cells were isolated from MM-PB or MM-BM by magnetic Ab selection or FACS sorting. Bone marrow stroma cells (BMSCs) were generated from fresh MM-BM. Cell growth was determined using 3H-thymidine incorporation or MTT assays. **Results.** Phenotypic analysis by flow cytometry showed a significant increase in $CD14^{-}CD11b^{+}HLA\text{-}DR^{low}CD33^{+}CD15^{+}$ MDSCs in fresh PB and BM from MM patients compared to healthy donors ($p<0.01$). MDSCs are highly heterogeneous cell populations, and they can be characterized by their tumor promoting, immunosuppressive functions. To determine whether the $CD14^{-}CD11b^{+}HLA\text{-}DR^{low}CD33^{+}CD15^{+}$ myeloid cell population represents functional MDSCs, we first assessed tumor promoting role of MDSCs in the MM microenvironment. MM-BMSCs were cocultured, with or without depletion of MDSCs with MM cell lines. When MDSCs were depleted from BMSC microenvironment, BMSC-mediated MM growth was significantly decreased to baseline levels of MM cells alone. Moreover, when isolated MDSCs from MM-BM were cultured with MM cell lines, there was a marked increase in MM cell growth, compared to MM cells alone. Since, the interaction between tumor and stromal accessory cells is bidirectional, we next analysed the impact of MM cells on MDSC development. MM cell lines cultured with PBMCs from healthy donors induced a 7 fold increase in MDSCs. Next, immune suppressive function of MDSCs was assessed in the culture of autologous T cells and isolated MDSCs from MM-PB or MM-BM. Importantly, freshly isolated MDSCs from both MM-PB and MM-BM induced significant inhibition of autologous T cell proliferation. Finally, we analysed the role of MDSCs in CAM-DR for bortezomib and lenalidomide. Culture of MDSCs with MM cell lines, with or without bortezomib (5nM) and lenalidomide (1uM), demonstrated that bortezomib induced less cytotoxicity against MM cells in the presence of MDSCs. **Summary and Conclusions.** Overall our preliminary data suggest that MDSCs are increased in MM microenvironment and play an important role in microenvironment mediated MM pathogenesis and immune suppression. Ongoing characterization of their direct impact on tumorigenesis, stromal tumor promotion, and in drug resistance will provide new therapeutic approaches in MM.

0274

IDENTIFICATION OF THERAPEUTIC CANDIDATE TARGETS SPECIFICALLY EXPRESSED IN MYELOMA SIDE POPULATION CELLS

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Background. Multiple myeloma (MM) is characterized by the accumulation of a population of malignant plasma cells within the bone marrow. MM is generally responsive to conventional chemotherapy followed by myeloablative doses of alkylating agents and autologous stem cell transplantation. Cytotoxic chemotherapy-based treatment is not curative, however, and the disease eventually recurs. Although currently available anti-MM strategies are effective at targeting the bulk of tumor cells, it is not clear that these agents are targeting the tumor-initiating subpopulation, including side population (SP) cells. SP cells express high levels of various members of the ABC transporter family, which are responsible for their drug resistance. A recent work by Jakubikova et al (2011) demonstrated that SP cells in MM have shown to exhibit stem cell like characteristics as well as high tumorigenicity. **Aims.** The aim of our study is to identify gene/proteins specifically expressed in myeloma SP cells, which could be essential therapeutic targets. **Methods.** We isolated SP cells from MM cell lines by use of Hechest 33342 dye to examine potential of tumorigenicity (transplantation SP cells into NOD/Shi-scid IL-2γnul mice). Gene expression analysis of SP and non-SP cells were performed against 5 MM cell lines (RPMI 8226, AMO1, KMS11, KMS-12BM and JJN3) and 8 primary samples (n=6 fresh samples, n=2 relapsed samples). Aurora kinase inhibitor (VX-680), 3-deazaneplanocin A(DZNep), and a proteasome inhibitor, bortezomib were exposed to RPMI 8226 and AMO1. **Results.** We found that myeloma SP cells expressing CD138 exhibit greater tumorigenetic potential than non SP cells. Gene expression analysis of 5 cell lines and CD138-positive primary samples revealed that, in SP cells, expression of cell cycle (e.g. *CCNB1, CDC25C, CDC2, CDC20, GTSE1*)-, microtubule attachment (e.g. *BIRC5, CENPE, SKA1)*-, mitosis or centrosome (e.g. *AURKB, KIF2C, KIF11, KIF15*)-, proliferation (e.g. *TOP2A, ASPM*)-, polycomb (e.g. *EPC1, EZH2*)- and ubiquitin-proteasome (e.g. *UBE2D3, UBE3C*, *PSMA5*)- genes was significantly stronger than in non-SP cells. On that basis, we used an aurora kinase inhibitor (VX-680 for AURKB), histone methylation inhibitor (DZNep for EZH2) and a proteasome inhibitor (bortezomib for S20/S26 proteasomes) against RPMI 8226 and AMO1. Of these, bortezomib reduced the SP fraction more effectively than the other agents due to its ability to reduce levels of phospho-histone H3, EZH2 and PSMA5. This suggests bortezomib has a greater range of targets than other agents and could include cell cycle, centrosome, polycomb and proteasome genes/proteins. **Conclusions.** We found that myeloma SP cells exhibit strong tumorigenicity *in vivo*, and showed that they more strongly express cell cycle (G_2/M)-, centrosome-, polycomb- and ubiquitin-proteasome-related genes than non-SP cells. We also demonstrated that reducing transcription of all these genes is necessary when targeting myeloma SP cells, and that bortezomib is capable of shrinking the myeloma SP by downregulating the aforementioned genes. Our approach could be useful for screening new agents with which to target a population possessing strong tumor initiating potential in MM.

0275

FUNCTIONALLY ACTIVE MYELOID-DERIVED SUPPRESSOR CELLS ARE INCREASED AFTER LENALIDOMIDE PLUS DEXAMETHASONE TREATMENT IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA PATIENTS

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Background. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature cells and reported to be expanded in pathological conditions including cancer, infectious diseases and some autoimmune diseases. Recently, a large number of studies showed MDSCs expansion and their suppressive function in tumor models and non-hematological malignancies. Multiple myeloma (MM) is a plasma cell malignancy associated with immunological impairments. **Aims.** To evaluate frequency of MDSCs and their suppressive function in MM patients at baseline and after lenalidomide plus dexamethasone (LD) treatment. **Patients and Methods.** A cohort of 16 untreated MM patients was included after obtaining signed informed consent forms according to Helsinki declaration. Median age of the studied cohort was 60 years (range, 36-67). According to international staging system (ISS) patients were staged as: ISS1, 8/16 (50%); ISS2, 5/16 (31%); and ISS3, 3/16 (19%). All patients received LD for 4 cycles as induction therapy (lenalidomide, 25mg on days 1-21; dexamethasone, 40mg on days 1, 8, 15, 22). Using multi-parameter flow cytometry (CD4-FITC, CD11b-PE, CD33-PerCpCy5.5, CD14-PECy7, HLA-DR-APC and CD8-PB), we assessed MDSCs, total lymphocytes, CD4 and CD8 T cells from PB of MM patients at baseline and after each LD treatment cycle. As controls, 11 healthy volunteers (HVs) PB samples were also assessed. Functional activity of MDSCs was studied using CFSE based T cell proliferation assays from 4 MM patients (3 untreated and 1 LD treated) and 3 HVs. Further, we profiled supernatants from proliferation assays for pro-inflammatory (IFN-γ, TNF-α) and immunosuppressive (IL-10) cytokines. **Results.** MDSCs were identified as CD33+CD11b+CD14-HLADR-. Frequency of MDSCs was significantly increased and total lymphocytes were reduced in MM patients at baseline compared to HVs (median% of MDSCs, 0.33 vs.0.08; P=0.001 and median% of lymphocytes, 16.10 vs. 36.13; P=0.01). However, baseline CD4 and CD8 T

cells from MM patients did not differ significantly from HVs. When we compared MDSCs from baseline and post-LD treatment, significant increase was observed after the 4th cycle but similar frequency was observed after 1-3 cycles (median% of MDSCs, 0.33 vs. 0.57; P=0.033). Functional studies revealed that MDSCs from MM patients and HVs suppressed CD3 T cell proliferation in concentration dependent manner (1:1, 1:5, 0:1). Suppressive function of MDSCs was comparable between HVs, untreated and LD treated patients. Cytokine data from MM and HVs showed that in the presence of MDSCs in proliferation assays, the level of IFN-γ, TNF-α and IL-10 was reduced significantly compared to proliferation assays without MDSCs (P=0.05). Comparison of MM and HVs cytokine data from proliferation assays (in presence or absence of MDSCs) showed no significant difference in the level of IFN-γ and TNF-α. But IL-10 level was 3-fold increased in proliferation assays (without MDSCs) from MM patients compared to HVs (mean pg/ml: 960.88 vs. 368.36; P=0.045). **Conclusions.** Our findings suggest that MDSCs are expanded and functional in MM. LD treatment in MM did not reduce MDSCs and their suppressive function. Expansion of these cells might enhance immune suppression in MM and thereby progress the disease.This study was supported by GACRGAP304/10/1395, MSM0021622434, LC06027, IGA: NS10406 and NS10408.

0276

MYELOMA PLASMA CELL CD45 NEGATIVITY CORRELATES WITH DRUG RESISTANCE AND MORE AGGRESSIVE BIOLOGY WHEN COMPARED TO CD45 POSITIVE COUNTERPARTS

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Background. Multiple myeloma (MM) is an incurable plasma cell (PC) malignancy predominantly negative for the protein tyrosine phosphatase CD45. Limited human data has demonstrated that the presence of proportionally greater numbers of $CD45^{-ve}$ rather than $CD45^{+ve}$ MM PCs confers shorter patient survival and murine data suggests that disease progression correlates with the transition from predominantly $CD45^{+ve}$ to predominantly $CD45^{-ve}$ disease. Based on these observations we hypothesised that CD45 negativity may be associated with increased drug resistance and a more malignant phenotype. **Aims.** To characterise and investigate the biology of MM PCs based on CD45 expression. **Methods.** U266 is an IL-6 secreting human MM cell line (HMCL) comprised of both $CD45^{+ve}$ and $CD45^{-ve}$ PC populations. These subsets were separated and analysed for differences in growth, surface phenotype, cytokine production, cell signalling, drug resistance and gene expression. **Results.** $CD45^{+ve}$ and $CD45^{-ve}$ PCs expressed similar levels of IL-6R, IGF-1R, CD49d and CD95, whereas the $CD45^{+ve}$ PCs expressed more CXCR4 and ICAM-1 (both $p<0.001$). Neither population produced IGF-1 and IL-6 was only produced by the $CD45^{-ve}$ population. Furthermore, upon IGF-1 stimulation, a 3-fold increase in IL-6 secretion ($p=0.04$) by the $CD45^{-ve}$ subset was observed. The $CD45^{+ve}$ cells displayed more rapid growth kinetics than the $CD45^{-ve}$ subset over 72hrs. But, comparable rates of $CD45^{-ve}$ PC growth were generated when the $CD45^{-ve}$ PCs were exposed to conditioned media (CM) from the $CD45^{+ve}$ PCs. Conversely, no change in growth was noted in the $CD45^{+ve}$ cells in the presence of CM from the $CD45^{-ve}$ cells. Basal p-AKT/p-STAT3/p-ERK expression was similar between the two subsets whereas both NOTCH and NFkB signalling was relatively increased in the $CD45^{-ve}$ PCs. Exposure to CM from the $CD45^{-ve}$ PCs resulted in a modest increase in p-AKT and p-ERK expression and a 3.5-fold increase in p-STAT3 in the $CD45^{+ve}$ subset whilst no change in signalling was detected in the $CD45^{-ve}$ PCs exposed to $CD45^{+ve}$ CM. The $CD45^{+ve}$ PCs exhibited a 2.9 fold and 2.1 fold increase, respectively, in bortezomib and marizomib induced cell death when compared to the $CD45^{-ve}$ PCs but no difference was observed following treatment with melphalan. Finally, comparative gene expression profiling ($CD45^{+ve}$ versus $CD45^{-ve}$) demonstrated increased expression of a range of tumour suppressor genes prone to promoter hypermethylation. Conversely, the $CD45^{-ve}$ PCs preferentially expressed a range of oncogenes known to be associated with metastasis and a more aggressive clinical phenotype in the context of solid tumour biology. **Conclusions.** Utilising the U266 HMCL as a model of CD45 expression we have demonstrated 1. co-operativity between $CD45^{+ve}$ and $CD45^{-ve}$ populations, suggesting that tumour heterogeneity within the context of MM may play a critical role in the promotion of the disease, and 2. that $CD45^{-ve}$ PCs exhibit increased drug resistance , increased activation of known oncogenic pathways and express a range of oncogenes known to be associated with metastasis and disease progression in solid tumours and that have not been previously described in MM.

0277

OSTEOCLASTOGENIC INHIBITORY PROPERTIES OF SMALL IMMUNOGLOBULIN COMPLEXES AND THEIR POTENTIAL AS A THERAPEUTIC FOR THE TREATMENT OF OSTEOLYTIC BONE DISEASE IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is concomitant with severe bone destruction. This destruction, driven by osteoclasts, is in part due to the osteoclastogenic properties of myeloma cells. However, this is not a unidirectional process but rather a complex inter-dependent process where osteoclasts also produce survival factors that support myeloma cell survival. Novel approaches targeting osteoclastogenesis in MM may therefore not only be beneficial for the treatment of osteolytic bone disease but also have an indirect inhibitory effect on tumour burden. Recent studies within our laboratory have shown that small immunoglobulin complexes of *Staphylococcal aureus* Protein A and Immunoglobulin G (SIC) can inhibit RANKL-driven osteoclastogeneis. **Aims.** To investigate the inhibitory potential of SIC on myeloma-driven osteoclastogenesis and the subsequent impact on myeloma cell survival. **Methods.** Primary myeloma cells were isolated from bone marrow aspirates of active, untreated MM patients using $CD138^{+}$ magnetic selection. Myeloma cells, or myeloma cell line U266B1, were co-cultured with human $CD14^{+}$ monocytes or pre-osteoclasts (M-CSF and RANKL cultured monocytes) in the presence or absence of SIC. Myeloma-driven osteoclastogenesis was assessed by tartrate-resistant acid phosphatase (TRAP) staining and osteolytic capacity by bone slice cultures. Myeloma cell survival was determined via flow cytometry. **Results.** To investigate whether or not SIC could inhibit osteoclastogenesis induced by myeloma cells we treated myeloma cell/pre-osteoclast co-cultures with SIC or relevant controls. The addition of SIC to co-cultures significantly inhibited the osteoclastogenic potential of both primary myeloma cells and the U266B1 human myeloma cell line. Interestingly, the source of osteoclast pre-cursors (allogenic or autologous) did not have an impact on the osteoclastogenic potential of myeloma cells or the inhibitory capacity of SIC. The osteolytic activity of cells in the presence of SIC, as determined by bone slice cultures, was also substantially reduced. This inhibitory effect was dose dependent and reversible.SIC binding studies revealed that SIC could not directly interact with myeloma cells. However, to determine whether the treatment of co-cultures could have an indirect effect on myeloma cell survival, due to alterations in the supportive environment, we evaluated the number of dead (Annexin V^{+} $7AAD^{+}$) myeloma cells after 7 days in co-culture in the presence or absence of SIC. The viability of U266B1 myeloma cells was markedly increased in the presence of pre-osteoclasts. The addition of SIC, however, abolished this supportive effect resulting in increased myeloma cell death. **Summary and Conclusions.** SIC directly interfere with myeloma-osteoclast interactions: inhibiting both myeloma-driven osteoclastogenesis and osteoclasts-driven myeloma cell survival. Thus, our data supports the idea that SIC might be a potentially novel therapeutic treatment strategy for multiple

0278

MICRO-RNA EXPRESSION IS ASSOCIATED WITH EXPRESSION OF DNA METHYLTRANSFERASES AND ANTI-APOPTOTIC GENE MCL-1 IN MULTIPLE MYELOMA AND MONOCLONAL GAMMMOPATHY WITH UNDETERMINED SIGNIFICANCE (MGUS)

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Background. Micro RNAs (miRs) are small non-coding RNAs of 19-25 bases in length having the ability to modulate the expression of other genes. MiRs are frequently involved in carcinogenesis and its expression analysis to predict the phenotype of malignancies has been shown. DNA methyltransferase (DNMT) is an enzyme that adds methyl group to cytosine of DNA keeping tumor suppressor genes (TSG) silent. It has been demonstrated that miR-15, miR-16, miR-29 family down-regulate DNMT and anti-apoptotic gene Mcl-1 expression then influence the survival of cancer cells *in vitro*. Many studies evaluate miR expression and its association with chromosomal abnormalities and the prognosis of multiple myeloma (MM), but the relationship between miR and its target gene expression in the patients has not been reported. **Aims.** In this study, we examine the relationship between miR-15, 16, 29 family expression and DNMT, Mcl-1 expression in MM and MGUS *in vivo* in patients. **Methods.** MM cell lines and BM samples obtained from 26 of MM patients, 16 of MGUS patients are subjected to the study after informed consent. Sequential BM samples during the treatment were obtained from 6 patients. RNA and miR were extracted from plasma cells separated by CD138 antibody and magnetic beads. MiR15a, 15b, 16, 29a, 29b, 29c and mRNA expression of DNMT1, 3A, 3B, Mcl-1 were quantified by Taqman-probe and real time PCR. **Results.** The expression level of miR15a and 16 was reduced in plasma cells of MM (6.4±3.1, 19.5±7.5) than of MGUS (34.8±15.4, 72.1±36.5) and of normal subjects(74.4±1.5, 25.5±12.9)(p=0.02, p=0.02). MiR 15b, 29a, 29b, 29c expressions were not significantly different among MM, MGUS and normal subjects. Conversely, DNMT1, 3A and Mcl-1 expression were elevated in MM (7.2±4.5, 8.5±3.3, 594±314) than in MGUS (0.8±0.2, 1.7±0.6, 41.4±41.2) and normal subjects (0.4±0.3, 2.1±1.8, 44.0±8.7) (p=0.03). DNMT3B expression was not different. In the MM cell lines, miR 29a and 29b expression were inversely correlated with DNMT1 mRNA expression (r=0.96, p=0.0003: r=0.86, p=0.014). In the patient samples, DNMT1 was inversely correlated with miR15a, miR15b, miR16, miR29a, miR29b (r=-0.435, p=0.003; r=-0.341, p=0.02, r=-0.332, p=0.03, r=-0.419, p=0.005, r=-0.407, p=0.006), DNMT3A was inversely correlated with miR15a, miR29a, miR29b, miR29c (r=-0.365, p=0.02; r=-0.315, p=0.04; r=-0.371, p=0.01; r=-0.315, p=0.04), DNMT3B was inversely correlated with miR15a, miR15b, miR29a, miR29b (r=-0.418, p=0.005; r=-0.385, p=0.01; r=-0.353, p=0.02; r=-0.358, p=0.02), and Mcl-1 was inversely correlated with miR29a (r=-0.028, p=0.03). Four of 6 patients' plasma cells showed decreasing miR 15a, 15b, 16 and 29 families and increasing DNMT1 and Mcl-1 expression during the treatment. Those patients acquired drug resistance. One patient's sample whose miR expression increased during the treatment showed reduced DNMT1 and Mcl-1 expression. **Conclusions.** We found that miRs decreased in MM and possibly associated with MM progression. Negative correlation with the expression level of the DNMTs, Mcl-1and miRs in MM cell lines and patients' samples were seen, which is consistent with previous reports about solid cancers. The sequential samples during the treatment suggest that miRs regulate its target gene expression *in vivo* in patients and thus related to MM prognosis.

0279

COMPREHENSIVE INVESTIGATION OF THE 8Q24 REGION AND MULTIPLE MYELOMA RISK IN THE CONTEXT OF THE IMMENSE (INTERNATIONAL MULTIPLE MYELOMA RESEARCH) CONSORTIUM

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Background. Recently, genome-wide association studies have identified and confirmed associations of glioma, chronic lymphocytic leukemia (CLL), breast, prostate, bladder, colorectal and ovarian cancer with several variants within a 600-Kb region of a longer, 1.18-Mb sequence, on chromosome 8q24. The 8q24 locus is devoid of genes, but telomeric to region 5 lies the MYC gene and a possible interaction among genetic variants of the 8q.24 region and MYC expression or activation have bas been reported. Since genetic factors are thought to be involved in Multiple Myeloma (MM) pathogenesis we postulated the hypothesis that variants in the 8q24 region could play a role also in MM risk. **Aims.** The aim of this study was to investigate whether genetic variants in the 8q24 region are associated with MM risk. **Methods.** We selected 20 SNPs spanning the 8q24 region and which showed associations in GWAS on CLL, breast, prostate, ovarian, bladder and colorectal cancer. The selected SNPs were genotyped in a population of 1188 MM cases and 2465 controls, ascertained through the IMMEnSE consortium and the Heidelberg Myeloma Group. **Results.** The carriers of the G allele of rs2456449 showed a statistically significant increased risk of MM ($OR_{carriers}$=1.21, 95%CI 1.05-1.40, P_{value}=0.009, P*trend*=0.030). Subdividing for recruitment center, in the population from Heidelberg, the observed association reached the study-wise significant threshold ($OR_{carriers}$=1.37, 95%CI 1.12-1.68, P_{value}=0.0022 P*trend*=0.0030) (Table 1).

Table 1. Distribution of genotypes of the 8q24 SNP rs2456449 in MM cases and controls.

8q24 SNP	Cases	Controls	OR*	95%C.I.*	P-value
rs2456449		IMMEnSE Consortium			
A/A	300 (48.6)	448 (50.4)	1	Ref.	-
A/G	238 (38.6)	350 (39.4)	1.01	0.81-1.26	0.928
G/G	79 (12.8)	91 (10.2)	1.30	0.93-1.83	0.123
A/G+G/G	317 (51.4)	441 (49.6)	1.07	0.87-1.32	0.518
				p-trend	0.223
rs2456449		Heidelberg Myeloma Group			
A/A	194 (34.7)	655 (42.1)	1	Ref.	-
A/G	278 (49.7)	697 (44.9)	1.35	1.09-1.67	***0.006***
G/G	87 (15.6)	202 (13.0)	1.45	1.07-1.95	***0.015***
A/G+G/G	365 (65.3)	899 (57.9)	1.37	1.12-1.68	***0.002***
				p-trend	***0.003***
rs2456449		Overall Population			
A/A	494 (42.0)	1103 (45.2)	1	Ref.	-
A/G	516 (43.9)	1047 (42.8)	1.17	1.00-1.36	***0.047***
G/G	166 (14.1)	293 (12.0)	1.37	1.10-1.71	***0.006***
A/G+G/G	682 (68.0)	1340 (54.8)	1.21	1.05-1.40	***0.009***
				p-trend	***0.030***

Genotype distribution among MM cases and controls in the IMMEnSE consortium, in the Heidelberg Myeloma Group and in the overall population. *OR=Odds Ratio, C.I.=Confidence Intervals. OR are adjusted for age, gender and region of origin. Differences in samples numbers are due to failures in genotyping. Results in bold show p<0.05.

Conclusions. We found a statistically significant association between the G allele of rs2456449 and an increased risk of MM. This association is intriguing because the rs2456449 has been shown to be associated with CLL and monoclonal B-cell lymphocytosis risk. The rs2456449 G allele could therefore represent a risk factor shared by several B-cell malignancies through a common regulatory mechanism. The observed effect of genetic variants within the 8q24 locus and Myc transcription/expression could represent the functional basis of the reported association. In conclusion these data provide further evidence that the genetic variability in the 8q24 region might be associated with cancer risk and in particular with B-cell malignancies.

Myeloma - Clinical 1

0280

ENHANCED ACTIVITY OF POMALIDOMIDE AND DEXAMETHASONE IN RELAPSED REFRACTORY MYELOMA IRRESPECTIVE TO EFFICACY IN THEIR LAST PRIOR LINE OF THERAPY. ON BEHALF OF IFM (INTER-GROUPE FRANCOPHONE DU MYÉLOME).

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Background. The IFM 2009-02 study aimed to determine the impact of the combination of pomalidomide (oral 4 mg daily) and dexamethasone (oral 40 mg weekly) in pts characterized with advanced myeloma (MM) resistant/refractory to lenalidomide and bortezomib. We have also analyzed the efficacy of pomalidomide and dexamethasone as compared to the respective last prior line of therapy. **Aims.** To compare the efficacy of pomalidomide and dexamethasone with that of the last prior line of therapy in patients with refractory myeloma.**Method.** This phase 2 multicenter study randomized MM patients who were non-responsive (≤ SD) or refractory (relapsed ≤ 60 days of last dose) to the last line of lenalidomide and/or bortezomib, as per IMWG criteria. Patients received pomalidomide 4 mg 21/28 days or 28/28 days plus dexamethasone. The primary objective was ORR (PR and better). All responses were reviewed by an independent committee (IRC). All patients had venous thromboembolism prophylaxis. All analyses are performed as ITT.

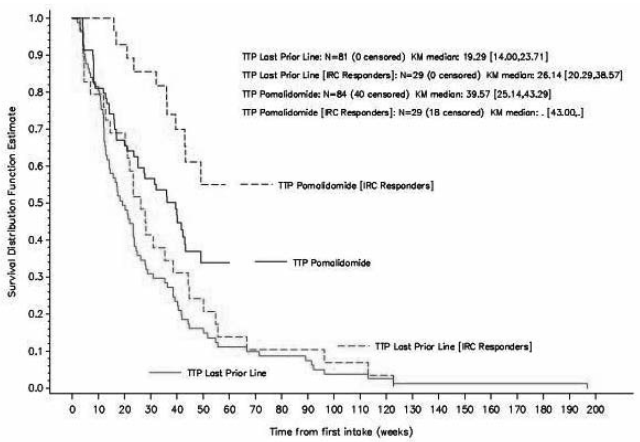

Figure 1. Comparison between TTP on-protocol and TTP on last prior line (ITT n=84).

Results. Eighty four patients (57 male and 27 female) were enrolled; 43 in arm 21/28 and 41 in arm 28/28. The median (min-max) age was 60 years (42-83). The median time from diagnosis to randomization was 70.5 months (9-277). The median number of prior lines of therapy was 5 (1-13), and 100% of the patients had received bortezomib and/or lenalidomide; 73% had received alkylating agents and thalidomide. As the last prior line of therapy, 38% had bortezomib-, 35% had lenalidomide-, 11% had bortezomib and lenalidomide-, 10% had thalidomide-, and 37% had an alkylating agent-based regimen. At the cut-off of March 1st 2011, the median follow-up for surviving pts was 10.4 months (2-14), the median number of cycles administered was 8 and6 in arm 21/28 and 28/28 respectively. The ORR was 35% in arm 21/28 and 34% in arm 28/28; 40% and 48% of patients had SD respectively. The median PFS was 6.3 months in both arms, and the median duration of response was 11.4 months and 7.9 months respectively. The median PFS was 3 times longer in responders versus patients that had a SD. Across both arms, patients refractory to the last line of therapy and to lenalidomide as the last prior line of therapy had a similar ORR and median PFS as compared to the entire study population, 32% and 23%, and 5.5 months (4-9) and 6.3 months (3-10), respectively. Regardless to whether patients responded to their last prior therapy, TTP on pomalidomide +dexamethasone was prolonged in the overall populations, from 5 months (3.5-6) to 10 months (6-11) and in patients who achieved a response, from 6.5 months (5-9.5) to NR (11—) (Figure 1). Updated efficacy and safety to be presented at EHA 2012. **Conclusions.** Pomalidomide and dexamethasone is active and generally well tolerated in heavily pre-treated patients with refractory multiple myeloma. This study provides further evidence that pomalidomide has no-cross resistance with lenalidomide and suggests that it can provide benefit for patients who have relapsed after other novel therapies including lenalidomide and bortezomib.

0281

VANTAGE 088: FINAL RESULTS FROM THE GLOBAL PHASE 3 TRIAL OF THE MULTI-HISTONE DEACETYLASE INHIBITOR VORINOSTAT IN COMBINATION WITH BORTEZOMIB IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background. The synergistic effects of vorinostat (VOR), an oral multi-histone deacetylase inhibitor, and bortezomib (BTZ) were shown in preclinical studies and confirmed in independent phase 1 trials in patients with relapsed/refractory multiple myeloma (MM). **Aims.** We conducted a global, randomized, placebo-controlled, phase 3 trial to investigate the benefits of VOR in combination with BTZ for the treatment of relapsed/refractory MM patients. **Methods.** Eligible patients (≥18 years, measurable MM, 1-3 prior regimens, ECOG ≤2) were randomized 1:1 to receive 21-day cycles of BTZ (1.3 mg/m^2 intravenously; days 1, 4, 8, 11) in combination with oral VOR (400 mg/d) or matching placebo, on days 1 to 14. Patients with prior resistance to BTZ were excluded, and additional use of corticosteroids for the treatment of MM was not allowed during the trial. All randomized patients were treated until disease progression, unacceptable toxicities, or withdrawal of consent. The primary end point was progression-free survival (PFS; after the occurrence of ~412 PFS events); all responses and progressions were determined according to the European Bone and Marrow Transplantation Group criteria and confirmed by an independent adjudication committee. **Results.** The trial enrolled 637 patients in 174 centers from 33 countries, making this trial one of the largest studies conducted in relapsed/refractory MM. The median age was 61 years (range 30-85 years), and patients had received a median of 2 prior regimens (range 1-3). Patients treated with VOR + BTZ compared with BTZ alone had a significant improvement in objective response rate (56% vs 41%; P<0.0001) and clinical benefit rate (71% vs 54%; P<0.0001). The durations of the induced responses were similar in both arms (8.5 vs 8.4 months); PFS and time to progression were signif-

icantly improved with VOR + BTZ compared with BTZ alone, with hazard ratio (HR) reductions of 23% (*P*=0.01) and 21% (*P*=0.02), respectively. With only 151 deaths, a favorable, nonsignificant trend in overall survival for VOR + BTZ was observed compared with BTZ alone (14% HR reduction; *P*=0.35). As expected, the most common treatment-emergent adverse events in both arms were predominantly hematologic and gastrointestinal disorders, with a significant increase for grade ≥3 thrombocytopenia (45% vs 24%; *P*<0.001) and grade ≥3 diarrhea (17% vs 9%; *P*=0.003) in the VOR + BTZ arm compared with BTZ alone. Mean exposure to study treatment was higher in the VOR + BTZ versus BTZ alone arm (7 vs 6 cycles); no differences in discontinuation rates were observed between the study arms (21% vs 22%, respectively). The combination was generally well-tolerated, and side effects were clinically manageable. Despite the increased incidence of grade ≥3 thrombocytopenia, no clinically relevant bleeding events have been reported. **Summary and Conclusions.** As the combination of VOR + BTZ induces significantly higher response rates and prolongs PFS in comparison with BTZ alone, VOR + BTZ may provide a new treatment option for relapsed/refractory MM patients. Subgroup analyses of high-risk patients will be presented at the meeting.

0282

ADVANCED AGE, ORGAN DAMAGE AND ADVERSE EVENTS NEGATIVELY AFFECT SURVIVAL OF MYELOMA PATIENTS RECEIVING NOVEL AGENTS: A META-ANALYSIS OF 1435 INDIVIDUAL PATIENT DATA FROM 4 RANDOMIZED CLINICAL TRIALS

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Background Multiple myeloma (MM) is the most relevant hematologic tumor in the elderly population. Median age at diagnosis is 70 years; 37% of patients are older than 75 years and 30% of patients present at diagnosis at least one co-morbid condition. The introduction of novel agents, bortezomib, thalidomide and lenalidomide, have changed the treatment paradigm of elderly MM, but side effects are frequent and full-drug doses less tolerated. Up to 90% of at least one serious adverse event (AE), with a subsequent 40% of drug discontinuation has been reported. **Aims.** A retrospective analysis evaluating 1435 individual patient data from 4 European phase III trials was conducted. We analyzed the impact of age, organ damage, treatment-related AEs and drug discontinuation as predictors of outcome. **Methods.** Patients with newly diagnosed MM, not eligible for autologous transplantation due to age (≥65 years) or co-morbidities, received melphalan-prednisone (MP), MP-thalidomide (MPT), MP-bortezomib (VMP), bortezomib-thalidomide-prednisone (VTP) or VMP-thalidomide (VMPT). Patients enrolled in the GISMM-2001 MP vs. MPT (331 patients), HOVON 49 MP vs. MPT (333 patients), GEM05MAS VMP vs. VTP (260 patients) and GIMEMA MM0305 VMP vs. VMPT (511 patients) trials were included in this meta-analysis. Trials were registered at ClinicalTrials.gov or controlled-trials.com. **Results.** Of the 1435 patients analysed, 332 did not receive novel agents (MP), 332 received thalidomide (MPT), 387 bortezomib (VMP), 384 thalidomide and bortezomib (VTP/ VMPT). Patients ≥75 years were 36%, equally distributed in the 4 groups. The proportion of patients with ISS III was lower in the MP group; renal failure was higher in the MP and MPT groups, due to less stringent selection of these protocols.The incidence of any grade 3-4 non-hematologic AEs was 29%, higher in MPT (43%) compared with VMP (24%) or VTP/VMPT (32%) groups. The most frequent were infections (10%), peripheral neuropathy (8%), cardiac (6%) and gastro-intestinal complications (5%). Drug discontinuation for toxicity was 27%, higher in MPT (35%) compared with VMP (16%) or VTP/VMPT (29%) groups.After a median follow-up of 33 months (95% Confidence Interval [CI] 10-56 months), 513/1435 patients (36%) died, the median overall survival was 50 months (95% CI 46-60 months). The causes of death were disease progression (76%) and toxic effects (24%), mainly infections, cardiac complications, second primary malignancies, and venous thromboembolism. The risk of death was increased in patients ≥75 years (Hazard Ratio [HR] 1.44, 95% CI 1.20-1.72, P<0.001), in patients with renal failure (HR 2.02, 95% CI 1.51-2.70, P<0.001), in those who experienced grade 3-4 cardiac, infective, or gastrointestinal AEs during treatment (HR 2.53, 95% CI 1.75-3.64, P<0.001) or requiring drug discontinuation due to AEs (HR 1.67, 95% CI 1.12-2.51, P=0.01). The risk of death increased in patients receiving more complex approaches with the combination of bortezomib-thalidomide. **Conclusions.** Age ≥75 years or renal failure at presentation, occurrence of cardiac, infective or gastrointestinal AEs negatively affected survival. An appropriate screening for vulnerability, assessment of organ function and less-intense personalized approaches are suggested in elderly unfit MM patients, to improve tolerability and optimize efficacy.

0283

SMOLDERING MULTIPLE MYELOMA AT HIGH-RISK OF PROGRESSION TO SYMPTOMATIC DISEASE: A RANDOMIZED TRIAL OF LEN-DEX AS INDUCTION FOLLOWED BY MAINTENANCE THERAPY WITH LEN ALONE VS NO TREATMENT

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Smoldering Multiple Myeloma (SMM) is an asymptomatic proliferative disorder of plasma cells (PCs) defined by a serum monoclonal component (MC) of 30 g/L or higher and/or 10% or more plasma cells in the bone marrow (BM). There are several risk factors predicting high-risk of progression to symptomatic disease: >10% of PCs in BM, serum MC >30g/L, >95% aberrant PCs by immunophenotyping, or abnormal free-light chains. Standard of care of SMM is no treatment until progression disease.In this phase III trial, SMM patients at high-risk of progression were randomized to receive Len-dex as induction followed by Len alone as maintenance vs no treatment in order to evaluate whether the early treatment prolongs the time to progression (TTP) to symptomatic disease.Len-dex arm received an induction treatment consisting on nine four-weekscycles of lenalidomide at dose of 25 mg daily on days 1-21 plusdexamethasone at dose of 20 mg daily on days 1-4 and 12-15 (totaldose: 160mg), followed by maintenance until progression disease withLenalidomide at dose of 10 mg on days 1-21 monthly up to two years.The 124 planned patients were already recruited, and 118 were evaluable (six patients didn't meet inclusion criteria).On an ITT analysis (n=57), based on IMWG criteria, the overallresponse rate during induction therapy was 81%, including 56% PR, 11% VGPR, 7% CR and7% sCR. 51 patients have completedthe nine induction cycles, and the ORR was 87%, including 12% VGPR, 8% CRand 8% sCR. After a median of 15 cycles of maintenance therapy (2-31), the sCR increased to 16%.After a median follow-up of 32 months (range: 5-42),nine patients progressed to symptomatic disease in the Len-dex arm: four of them during maintenance therapy and the other five progressed after early discontinuation of the trial due to personal reasons. In the therapeutic abstention arm, 37 out of 61 patients (59%) progressed to active MM. The estimated hazard ratio was 6· 0 (95%CI= 2· 9-12.6), corresponding to a median TTP from inclusion of 23

monthsfor the not treatment arm vs median not reached in the treatment arm (p<0.0001). Estimated 3-years overall survival (OS) from the inclusion in the trial was 93% for Len-dex arm and 76% for no treatment arm (p=0· 04) and this difference was more evident if we evaluate the OS from the moment of diagnosis (HR: 5; 95% IC (1-22); p=0.03).As far as toxicity is concerned, during induction therapy, only one infection G4 was reported. The G3 adverse events (AEs) most frequently reported were: anemia (1), asthenia (4), diarrhea (1), skin rash (2) and infections (4).In conclusion, this analysis shows that in high-risk SMM patients, early treatment with Len-dex as induction followed by Len as maintenance significantly prolonged the TTP to symptomatic disease (HR: 6· 0), resulting into a benefit in overall survival. In addition, tolerability is acceptable. Moreover, biological progressions occurring under maintenance have remained controlled over a prolonged period of time.

0284

EXTENDED TREATMENT WITH THE COMBINATION OF CARFILZOMIB (CFZ), LENALIDOMIDE (LEN), AND DEXAMETHASONE (DEX) IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM)

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Background. We have previously reported safety and efficacy data of the combination of CFZ, LEN (Revlimid®), and low-dose DEX (CRd) from a phase 1/2 study of patients with NDMM (Jakubowiak et al. ASH 2011, Abstract 631). CRd provided rapid reduction of disease (68%) after cycle 1, and after a median of 8 cycles, 94% of patients achieved ≥partial response (PR), including 65% with ≥very good PR and 53% with ≥near complete response (nCR). In patients who received at least 12 cycles of CRd, the rate of ≥nCR improved to 79%. The regimen was well tolerated. **Aims.** To examine the clinical impact of prolonged CRd treatment, including maintenance therapy. **Methods.** Patients with NDMM were treated in 28-day cycles with CFZ 20-36 mg/m^2 IV (d1, 2, 8, 9, 15, 16), LEN 25 mg PO (d1-21), and DEX 40/20 mg (cycles 1-4/5-8) PO weekly. After cycle 4, autologous stem cell transplant (ASCT) candidates achieving ≥PR could undergo stem cell collection but then continued CRd with the option to proceed to ASCT. After cycle 8, patients continued CRd on a maintenance schedule using the last tolerated doses, with LEN/DEX at the same schedule but a modified CFZ schedule (d1, 2, 15, 16). Response was assessed by IMWG criteria with the addition of nCR. **Results.** As of November 30, 2011, 53 patients were enrolled with a median follow-up of 14 months (range 4-25) and a median number of treatment cycles of 13 (range 1-25). Overall, 33 (62%) patients achieved ≥nCR with a stringent CR (sCR) rate of 42%. The rate of sCR improved with the duration of treatment. At the completion of 4 cycles, 41% of patients were in ≥nCR with only 6% in sCR. Of 36 patients who completed 8 cycles and continued with CRd maintenance, 72% were in ≥nCR with 64% in sCR. Minimal residual disease (MRD) was assessed by multiparameter flow cytometry in 22 patients in CR with no evidence of MRD in 20 (91%) of these patients. The progression-free survival (PFS) rate was 97% at 12 months and 92% at 24 months. All patients who achieved sCR have maintained response for a median of 9 months (range 1-20). Extended treatment with CRd was well tolerated. During CRd maintenance, the most common toxicities (all grades) were lymphopenia (30%), leukopenia (26%), and fatigue (25%). Peripheral neuropathy was infrequent (11%) and mild to moderate in severity (grades 1/2). There were no treatment discontinuations due to toxicity during maintenance and limited dose modifications (CFZ 19%, LEN 28%, DEX 31%).**Summary and Conclusions.** Data from this study indicate that after a rapid initial response, prolonged CRd treatment steadily improves the depth of response with most patients eventually achieving sCR and a significant proportion of patients without evidence of MRD. Responses were durable with very promising PFS. These results compare favorably to other frontline regimens and support further development of CRd in this setting.

0285

GMMG MM5 TRIAL IN NEWLY DIAGNOSED MULTIPLE MYELOMA TO EVALUATE PAD VS VCD INDUCTION PRIOR TO HDT FOLLOWED BY LENALIDOMIDE CONSOLIDATION AND MAINTENANCE - FIRST INTERIM ANALYSIS ON INDUCTION

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Background. The MM5 phase III trial of the German-Speaking Myeloma Multicenter Group (GMMG) was designed to address two independent primary objectives: 1.) Demonstration of non-inferiority of VCD (bortezomib, cyclophosphamide, dexamethasone) induction compared to PAd (bortezomib, adriamycin, dexamethasone) induction therapy with respect to response rate (very good partial response or better). 2.) Determination of the best of four treatment strategies with respect to progression-free survival (PFS). The four treatment strategies are defined by PAd vs. VCD induction treatment, high dose melphalan followed by autologous stem cell transplantation and maintenance treatment with lenalidomide for 2 years vs. lenalidomide until complete response (CR). During the induction phase the patients are treated with 3 cycles of PAd or VCD. PAd was dosed as bortezomib 1.3 mg/m^2, days 1, 4, 8, 11, doxorubicin 9 mg/m^2, days 1-4, dexamethasone 20 mg, days 1-4, 9-12, 17-20 (repeated every 28 days). VCD consisted of bortezomib 1.3 mg/m^2, days 1, 4, 8, 11, cyclophosphamide 900 mg/m^2 day 1, dexamethasone 40 mg, days 1-2, 4-5, 8-9, 11-12 (repeated every 21 days). **Methods.** An interim analysis with respect to response rates after induction treatment and a safety analysis were done in the first 150 patients (75 per regimen) as described in the protocol. Responses were assessed according to the response criteria of the International Myeloma Working Group, IMWG. **Results.** Patients treated with PAd or VCD were equally distributed for ISS and Durie-Salmon disease stage, age, kidney function and the cytogenetic abnormalities translocation t(4;14) and gain 1q21. Deletion17p13 was found more frequently in patients of the PAd group (17.1% vs. 6.1%). Seventy-one of 75 patients (94.7%) in the PAd group and 74 of 75 patients (98.7%) in the VCD group completed induction treatment. Response rate was similar in both induction regimens (PAd vs. VCD) with complete response (8% vs 9.3%), ≥ very good partial response (40% vs. 34.7%) and ≥ partial response (78.7% vs. 78.7%). The proportion of patients with any adverse event was comparable in PAd versus VCD (66.2% vs. 69.3%), but more serious adverse events (SAEs) were observed during PAd induction (32.4% vs. 22.7%). VCD led to a significantly higher proportion of leukocytopenia and neutropenia CTCAE °3 and 4 (PAd: 9.5% vs. VCD: 40%). The number of infections (≥ CTCAE °2) was higher during PAd induction (31.1% vs. 13.3%). Compared to the infection rate (≥ CTCAE °2) of 49% during PAD (dexamethasone 40 mg days 1-4, 9-12, 17-20) in the HOVON65/GMMG-HD4-trial, a reduction in MM5 during induction is observed. **Conclusions.** Both induction treatments in the current MM5 trial show relevant efficacy and no early non-inferiority of VCD compared to PAd was shown. PAd and VCD are well tolerated with more than 90% of the patients receiving the three planned induction cycles.

0286

LONG-TERM FOLLOW-UP OF PATIENTS WITH MULTIPLE MYELOMA RECEIVING BISPHOSPHONATES IN CONJUNCTION WITH ANTIMYELOMA THERAPY: MRC MYELOMA IX STUDY

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Background. Bisphosphonates (BP) are a standard of care for patients with

osteolytic lesions from multiple myeloma (MM). The MRC Myeloma IX study revealed significant overall survival (OS) and progression-free survival (PFS) benefits for the intravenous amino-BP, zoledronic acid (ZOL) over oral clodronate (CLO) in patients with MM (N = 1960) initiating chemotherapy (Morgan GJ, et al. *Lancet.* 2010). This trial was designed to include BP therapy at least until disease progression, and may provide insights into long-term effects of BP treatment as well as discontinuation of BPs before or upon disease progression. **Aims.** To assess the potential effects of discontinuing BP (ZOL) treatment on PFS and OS in patients with MM. **Methods.** Newly diagnosed MM patients were randomized to ZOL (4 mg IV q 21-28 days) or CLO (1600 mg/day PO) plus antimyeloma therapy. BP therapy was to be continued at least until disease progression. PFS and OS were estimated using Kaplan-Meier methodology. Hazard ratios (HR) were calculated using stratified Cox models. Exploratory post hoc Cox regression models including BP discontinuation as a time-dependent variable were used to assess potential correlations between stopping BP treatment on-study and long-term PFS or OS in ZOL-treated patients. **Results.** The MRC Myeloma IX trial now has a median follow-up of 5.9 years in 1960 evaluable patients. Median duration of BP treatment was 380 days (interquartile range, 153-716 days) before disease progression. BP therapy was discontinued before documented disease progression in 272 patients (28%) in the ZOL group and 204 patients (21%) in the CLO group. Notably, rates of treatment discontinuation for renal insufficiency or renal failure were low and similar across groups (~5% in each). Disease progression or death was documented in 856 patients (87%) in the ZOL arm vs 867 patients (89%) in the CLO arm. Relative improvements in PFS and OS with ZOL vs CLO were 11% (HR = 0.89; *P* = .02) and 14% (HR = 0.86; *P* = .01) respectively. Trends for improved PFS and OS (from baseline and after disease progression) with ZOL vs CLO were maintained in patients receiving BPs for ≥2 years (n = 539) but were not statistically significant. Exploratory Cox models including discontinuation of BP as a time-dependent variable suggest that stopping BP therapy did not affect PFS (*P* = .88) or OS (*P* = .42) in the ZOL arm. **Conclusions.** PFS and OS improvements with ZOL vs CLO (from baseline and after documented disease progression) were maintained during long-term follow-up in the MRC Myeloma IX study. Exploratory analyses in the ZOL arm suggest that on-study discontinuation of BP therapy did not significantly affect outcomes, but should be interpreted with caution because the trial did not collect BP discontinuation data after disease progression (ie, for these exploratory analyses, all patients receiving BPs at the time of first documented disease progression were assumed to have continued BP treatment until death). Further studies are required to determine the optimal duration of BP treatment.

0287

PHASE I/II STUDY OF CARFILZOMIB PLUS MELPHALAN-PREDNISONE (CMP) IN ELDERLY PATIENTS WITH *DE NOVO* MULTIPLE MYELOMA

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Background. Melphalan-Prednisone + Thalidomide (MPT) or Bortezomib (MPV) are approved in frontline MM patients (pts) >65 years. Both regimens demonstrated significant benefit over MP alone in terms of PFS and OS but this benefit could be hampered by the risk of peripheral neuropathy (PN). Carfilzomib (Cfz) is a novel proteasome inhibitor that has demonstrated promising activity and favorable toxicity profile, with low rates of PN. This phase I/II study was designed to determine the maximum tolerated dose (MTD) of CMP, to assess safety and evaluate efficacy of this combination in newly diagnosed MM >65.Methods: In Phase I, Cfz was the only escalating agent starting at 20 mg/m2 (level 1) with maximal planned dose 36 mg/m2 (level 3), given IV on days 1, 2, 8, 9, 22, 23, 29, 30 for nine 42-day cycles. Oral melphalan 9 mg/m² and prednisone 60mg/m² were given on days 1 to 4, for all dose levels. Based on toxicity assessment, the study was amended to add dose level 4 (Cfz 45 mg/m2). MTD determination was based on occurrence of Dose limiting toxicities (DLTs) during the first cycle only. DLTs were defined as any grade 4 hematologic toxicity or preventing administration of 2 or more of the 8 Cfz doses of the first treatment cycle except grade 4 thrombocytopenia without bleeding or grade 4 neutropenia lasting ≤ 7 days; or grade ≥ 3 febrile neutropenia; or any other grade ≥ 3 nonhematologic toxicity. **Results.** As of February 12th 2012, 24 pts have been enrolled in the phase I: 6 pts at level 1 (Cfz 20), 6 at level 2 (Cfz 27), 6 at level 3 (Cfz 36), and 6 at level 4 (Cfz 45). There were 2 DLTs at level 4 (fever and hypotension not related to sepsis) and the MTD was considered to be 36 mg/m². Then, 16 additional pts were included in the phase II at level 3. Overall, 40 pts have been enrolled into the phase I/II study. The protocol is ongoing, and results will be updated during the meeting. In the current report, the median number of cycles analyzed is 5 (2-9), and 28 patients are still on therapy. Thus, all patients received at least 2 cycles and were considered evaluable for response. The ORR was 92% including 40% at least VGPR. These results, probably underestimated due to the dose-escalation design of the study and ongoing therapy, compare favorably to those achieved with MPV, MPT, MPR or lenalidomide-dex (ORR 71, 76, 80 and 85%, respectively) in the same population of patients. **Conclusions.** Frontline carfilzomib (36 mg/m^2) + MP is a tolerable and very effective combination in elderly MM pts. Treatment is ongoing, with updated toxicity and efficacy data to be presented at the meeting.

0288

WITHDRAWN

0289

HIGH RESPONSE RATES TO POMALIDOMIDE-CYCLOPHOSPHAMIDE-PREDNISONE (PCP) IN PATIENTS WITH MULTIPLE MYELOMA RELAPSED/REFRACTORY TO LENALIDOMIDE: RESULTS OF A PHASE I/II STUDY

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Background. Patients with multiple myeloma (MM) relapsed/refractory after bortezomib and lenalidomide have limited treatment options and poor outcome. Pomalidomide and dexamethasone showed significant activity in this setting. The efficacy and safety of Pomalidomide in combination with Cyclophosphamide is unknown. **Aims.** This phase I/II study aimed to evaluate the safety and efficacy of Pomalidomide-Cyclophosphamide-Prednisone (PCP) in relapsed/refractory MM patients. **Methods.** We evaluated the maximum tolerated dose (MTD) and efficacy of Pomalidomide (4 dose levels: 1, 1.5, 2 and 2.5 mg/day) in combination with Cyclophosphamide (50 mg every other day) and Prednisone (50 mg every other day) for six 28-day cycles, followed by Pomalidomide-Prednisone as maintenance. Dose Limiting Toxicities (DLTs) were: grade 4 neutropenia>3 days, grade 4 thrombocytopenia, any grade 3-4 non-hematologic adverse events (AEs). The MTD was the dose that achieved a DLT in 25% of patients, according to the Bayesian Continual Reassessment Method. Patients who received 1-3 prior lines of therapy and were relapsed/refractory to Lenalidomide were eligible. All patients received aspirin or low-molecular weight heparin as thromboprophylaxis. **Results.** A total of 41 patients were enrolled. DLTs occurred in 1 patient at 1.5 mg (grade 4 thrombocytopenia) and in 3 patients at 2.5 mg (grade 3 neuropathy, grade 3 hepatic toxicity and grade 4 thrombocytopenia). The MTD was 2.5 mg with an estimated DLT probability of 0.258 (95% credibility interval: 0.101-0.468). Overall, 29 patients received 2.5 mg. Twenty-nine patients were evaluable. The median age was 69 years (41-80), 45% had ISS 2-3 and 21% was at high risk by FISH (presence of at least one of the following: t(4;14) or t(14;16) or del 17p13). Median time from diagnosis was 64 months. Patients had received a median of 3 prior regimens, including Lenalidomide (100%), Bortezomib (72%), Thalidomide (17%), stem cell transplant (38%). A partial response or better (ORR) was reported in 72% of patients, including 21% very good partial response and 7% complete response. A clinical benefit (ORR + minimal response + stable disease) was observed in 97% of the patients, including 8% of minimal response and 17% of stable disease. The ORR for patients who received ≤2 prior ther-

apies was 90%. In responding patients, median time to ORR was 2 months (range 1-6). After a median follow-up of 7.3 months, 24/29 patients (82%) are free of disease progression and 27/29 (93%) are alive. Toxicity consisted primarily in myelosuppression. Grade 4 AEs included neutropenia (17%), thrombocytopenia (7%) and thromboembolism (3%); grade 3AEs were rash (10%), peripheral neuropaty (3%), infections (3%). Discontinuations for toxicity were 10% (grade 3 hepatic, grade 4 central neurologic toxicity and thromboembolism). **Conclusions.** Pomalidomide in combination with Cyclophosphamide and Prednisone showed clinical activity with limited toxicities in patients relapsed/refractory to Lenalidomide. Updated data will be presented at the meeting.

0290

ESCALATED WEEKLY DOSE OF BORTEZOMIB WITH LENALIDOMIDE AND DEXAMETHASONE FOLLOWED BY LENALIDOMIDE MAINTENANCE IN FIRST RELAPSE OF MULTIPLE MYELOMA. INTERIM ANALYSIS OF THE HOVON 86 TRIAL

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Background. The combination of bortezomib (1.3 mg/m^2) and lenalidomide (10-25 mg) with dexamethasone (VRD) is effective in newly diagnosed and relapsed multiple myeloma (MM). Lenalidomide maintenance has been shown to prolong progression-free survival (PFS) but not overall survival (OS). **Aims.** This investigator-sponsored two-step phase 2 trial was designed to evaluate escalating dosages of bortezomib (B) given once weekly and daily lenalidomide (L) combined with weekly dexamethasone (D) (eVRD) followed by L maintenance in patients with first relapse MM. **Methods.** Dose levels were B 1.3 mg/m^2, L 10 mg, (level 1); B 1.6 mg/m^2, L 10 mg (level 2); B 1.6 mg/m^2, L 15 mg (level 3); B 1.6 mg/m^2, L 20 mg (level 4). Dexamethasone was dosed 20 mg days 1-2, 8-9, 15-16. Patients with symptomatic MM, aged 18-80 in first relapse after initial treatment were eligible. The primary endpoint was response (overall response (ORR), complete response (CR) according to IMWG criteria, serum free lite-negative CR (sCR), very good partial response (VGPR) and partial response (PR)) with PFS, OS and toxicity as secondary endpoints. **Results.** Eighty-one patients were included, i.e. 13 patients in dose levels 1, 2 and 3, followed by 68 patients in the phase 2 part. This interim analysis was performed on 13 patients in the dose escalation phase and 22 patients in phase 2. Median age was 66 years, with 62/33/5 per cent presenting with ISS stages I/II/III, respectively.The Maximum Tolerated Dose (MTD) was reached at dose level 3 when the maximum of 3 SAEs in 5 patients was observed. The second phase of the trial was performed at MTD minus 1 dose level, i.e. with weekly B 1.6 mg/m^2 for 3 weeks, L 10 mg days 1-21 and D 20 mg days 1-2,8-9,15-16 for 8 cycles of 28 days followed by L maintenance 10 mg days 1-21. The median number of cycles was 6 in the dose-escalation phase and 7 cycles in phase 2. 7/13 (62%) patients in the dose-escalation and 13/22 (59%) patients in phase 2 started lenalidomide maintenance. Full dose B/L was administered in 102/139(73%) and 125/139(90%) of cycles. Non-hematological toxicity included peripheral neuropathy (PNP) grade 2 in 14% of patients and grade 3 in 18%. The median time to first occurrence of PNP was 92 days. Gastro-intestinal symptoms grade ≤2 were observed in 36% of patients.At a median follow-up of 12.3 months, the overall response rate was 82% with 39% CR/nCR, 18% VGPR and 25% PR. Median time to response was 1.1 cycle. Actuarial PFS at 12 months was 70% and OS was 82%. Four patients died from MM and 2 from other causes. One second primary tumor was observed in dose level 3. FISH data are being completed to assess relative risks. **Summary and Conclusions.** Escalated VRD followed by Lenalidomide maintenance is effective and feasible in patients with first relapse MM. Responses are induced rapidly. This trial was registered as Eudract nr 2007-002533-37. Financial support was provided by the Dutch Cancer League KWF, Janssen and Celgene.

0291

PANORAMA 1: A PHASE III STUDY OF PANOBINOSTAT IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA

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Background. The development of novel agents, including the proteasome inhibitor bortezomib, has resulted in dramatic improvements in survival for patients with multiple myeloma (MM). However, MM remains an incurable disease, and there continues to be a significant unmet need in the relapsed and refractory setting. Thus, there is considerable interest in the discovery of effective agents and combination strategies for patients with relapsed and refractory MM. Panobinostat is an oral pan-deacetylase inhibitor with synergistic antimyeloma activity in combination with bortezomib, which may occur through dual inhibition of both the aggresome and proteasome pathways. A phase Ib study of panobinostat and bortezomib demonstrated clinical activity (≥ minimal response) in 40 of 62 (64.5%) patients with relapsed or relapsed/refractory MM, with a recent phase II study confirming this activity in bortezomib refractory MM. **Aims.** The primary objective of PANORAMA 1, a global, randomized, double-blind, phase III study of panobinostat + bortezomib + dexamethasone versus placebo + bortezomib + dexamethasone, is to evaluate the impact of panobinostat on progression-free survival in patients with relapsed MM. Secondary endpoints include overall survival, response rate, safety, and quality of life. Here, we report the patient demographics and the updated results of a blinded safety analysis from this study. **Methods.** Patients (N = 762) with relapsed or relapsed/refractory MM (1-3 prior lines of therapy) have been enrolled in the trial. Patients with bortezomib-refractory MM were not eligible. The study is composed of 2 treatment phases and follow-up during and after treatment until disease progression. Treatment phase 1 consisted of eight 3-week cycles of panobinostat or placebo (20 mg, oral) administered thrice weekly and bortezomib (1.3 mg/m^2, intravenous) administered twice weekly for 2 of 3 weeks. Dexamethasone (20 mg, oral) was administered on days of and after bortezomib. Patients who achieved clinical benefit proceeded to treatment phase 2, which consisted of four 6-week cycles with similar administration of panobinostat and dexamethasone and a modified once-weekly bortezomib schedule. **Results.** Preliminary demographic and blinded safety data from the first 536 randomized patients and 525 treated patients are now available, with treatment ongoing in 257 patients (47.9%). Median age was 63 years (32-84 years). Approximately half of patients (51.7%) received 2-3 prior lines of therapy and 57.3% received prior stem cell transplant. Adverse events of any grade included thrombocytopenia (47.6%), diarrhea (45.7%), fatigue (32.6%), anemia (30.5%), constipation (24.8%), and nausea (24.4%). Common grade 3/4 adverse events were thrombocytopenia (36.2%), diarrhea (14.5%), anemia (13.0%), fatigue (12.2%), neutropenia (11.4%), and hypokalemia (11.0%). Of

interest, peripheral neuropathy was observed in 24.6% of patients at any grade and 5.3% of patients at grade 3/4. **Conclusions.** Preliminary review of blinded pooled safety data in 525 patients treated in PANORAMA 1 suggested a comparable safety profile with respect to previously published clinical experience with bortezomib + dexamethasone. The data monitoring committee recommended that the study continue as planned. Enrollment of 762 patients was completed in February 2012. Updated blinded safety data will be presented.

0292

CLINICAL AND MOLECULAR ANALYSIS OF BORTEZOMIB- AND THALIDOMIDE-INDUCED PERIPHERAL NEUROPATHY (PN) IN MULTIPLE MYELOMA (MM): ANALYSIS FROM THE GIMEMA-MMY-3006 TRIAL

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Background. PN is an important complication of MM and its incidence has been further increased after the introduction of the novel agents thalidomide and bortezomib. **Aims.** We performed a subanalysis of the phase 3 GIMEMA-MMY-3006 trial aimed to assess clinical and molecular features of treatment-emergent PN, in newly diagnosed MM patients treated with Thalidomide-Dexamethasone (TD) or Bortezomib-TD (VTD) as induction therapy prior to, and consolidation after, double autologous stem-cell transplantation. **Methods.** The primary adverse event endpoint was treatment-emergent PN of at least NCI CTCAE grade 2, since grade 1 PN may be misinterpreted and does not interfere with daily activities. Overall, 474 patients (236 randomized to VTD and 238 to TD) were stratified according to the development of grade ≥2 PN. Gene expression profiles (GEP) of pre-treatment CD138+ bone marrow plasma cells were analyzed in a subset of 122 VTD-treated patients (44 with and 78 without treatment-emergent grade ≥2 PN). **Results.** Occurrence of PN was higher in VTD arm compared to TD. In particular, the rate of grade ≥2 PN was 35% vs 10% ($p<0.001$), and grade ≥3 was 15% vs 2.5% ($p<0.001$), respectively. The median time to onset of grade ≥2 PN was 83 days on VTD and 37.5 days on TD ($p=0.04$) and most of the events occurred during the induction phase (52% on VTD and 71% on TD). PN resolved in 88% of patients on VTD and 95% on TD within a median time of 73 and 61 days, respectively. In both arms, development of grade ≥2 PN did not adversely affect rates of complete or near-complete response, time to progression and progression free survival. By univariate analysis, baseline patient characteristics, including age, MM isotype, ISS stage, cytogenetic abnormalities, and pre-existing neuropathy, were not predictive for treatment-induced PN in both arms. By GEP analysis, grade ≥2 VTD-induced PN was characterized by 1461 differentially expressed probe sets ($p<0.05$, one-way ANOVA Partek Genomic Suite, version 6.4), 690 up-regulated and 771 down-regulated. The genes with the highest changes in expression arrays included genes that are etiologically linked to the development and function of the nervous system such as *NRN1* and *BASP1*. GeneGO® pathway analysis of differentially expressed genes showed enrichment of genes mainly implicated in cytoskeleton remodeling (*MAP3k1*, *XIAP*, *ACTA2*), and clathrin-coated vesicle cycle (*VAMP7* and *AP2A2*). Moreover, analysis of biological networks showed involvement of genes implicated in regulation of cytoskeleton rearrangement, neurogenesis and axonal guidance (*TNK2, ARHGAP4, ARGEF12, ACTA2, SEMA6A, SEMA4D, EPHA5, NEFH, DCTN1, DCX*). **Conclusions.** VTD incorporated into double ASCT was associated with a higher incidence of PN compared with TD. Treatment-emergent PN was reversible in the majority of patients in both arms and did not adversely affect treatment efficacy and outcomes. No relationship between development of PN and both patient demographics and disease characteristics was observed. Conversely, GEP results provide new insight into potential molecular mechanisms underlying VTD-induced PN and suggest a correlation with a myeloma genetic profile characterized by the significant deregulated expression of genes involved in the cytoskeleton rearrangement and nervous system development and function.

0293

ORAL MLN9708, AN INVESTIGATIONAL PROTEASOME INHIBITOR, IN COMBINATION WITH MELPHALAN AND PREDNISONE IN PATIENTS WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA: A PHASE 1 STUDY

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Background. MLN9708 is an investigational oral, potent, reversible, and specific 20S proteasome inhibitor undergoing clinical investigation for the treatment of hematologic malignancies and solid tumors. The proteasome inhibitor bortezomib in combination with melphalan plus prednisone (MP) is recommended for patients with previously untreated multiple myeloma (MM) that are not eligible for transplant. Here we report phase 1 results of the first study of MLN9708 in combination with MP in patients with previously untreated MM (NCT01335685). **Aims.** To determine the safety, maximum tolerated dose (MTD), recommended phase 2 dose (RP2D), pharmacokinetics (PK), pharmacodynamics (PD), and anti-tumor activity of MLN9708 administered once- or twice-weekly in combination with MP in patients with previously untreated MM. **Methods.** Adults with previously untreated MM with measurable disease but ineligible for transplant were enrolled. All patients provided informed consent. Patients received twice-weekly (TW) or weekly (W) oral MLN9708: TW schedule, MLN9708 on days 1, 4, 8, 11, 22, 25, 29, and 32, and MP (9 mg/m^2 and 60 mg/m^2, respectively) days 1-4 of 42-day cycles; W schedule, MLN9708 on days 1, 8, and 15 and MP (6 mg/m^2 and 60 mg/m^2, respectively) days 1-4 of 28-day cycles. Dose-escalation proceeded from a fixed dose of 3 mg in both schedules using a standard 3+3 schema based on the occurrence of dose-limiting toxicities (DLTs) in cycle 1. **Results.** At data cut-off (Jan 27, 2012), 13 patients had been enrolled and included in the safety population, 7 to the TW (3 mg and 3.7 mg dose levels), and 6 to the W (3 mg and 4 mg dose levels) schedules. Median age was 78 years (range 65-84) and 73 years (range 66-81) for the TW and W schedules, respectively; 29% and 50% were male, and 71% and 83% had ISS stage I/II disease. Patients are ongoing on treatment, with a median duration of treatment of 2.2 cycles for the TW and 3 cycles for the W schedule at time of data cut-off. One patient receiving 3.7 mg TW experienced DLTs of subileus and grade 3 rash. The MTD for MLN9708 has not been reached for either schedule. Safety data are shown in the Table 1.

Table 1.

	MLN9708 TW (n=7)	MLN9708 W (n=6)
Drug-related AEs, n (%)	7 (100)	6 (100)
Most common drug-related AEs, n (%)		
Neutropenia	4 (57)	4 (67)
Thrombocytopenia	4 (57)	4 (67)
Pyrexia	3 (43)	1 (17)
All-cause grade ≥3 AEs, n (%)	7 (100)	4 (67)
Drug-related grade ≥3 AEs, n (%)	6 (86)	2 (33)
Most common drug-related grade ≥3 AEs, n (%)		
Neutropenia	3 (43)	2 (33)
Thrombocytopenia	2 (29)	0 (0)
Serious AEs, n (%)	3 (43)	2 (33)
Discontinuations due to PD, n (%)	1 (14)	0 (0)
On-study deaths, n	0	0

Most common drug-related grade ≥3 adverse events (AEs) were neutropenia and thrombocytopenia; neutropenia occurred in 5 patients (3 on the TW schedule and 2 on the W schedule) and thrombocytopenia in 2 patients (both on the TW schedule). Three TW patients experienced drug-related serious AEs of neutropenia, subileus, vomiting, and pneumonia; 2 patients had serious AEs with W MLN9708, both unrelated. Thus far, of 6 TW response-evaluable patients, 4 patients achieved ≥PR (2 at each dose level, including 1 CR). Of 5 W response-evaluable patients, 3 achieved a PR. Dose escalation is ongoing.

Updated data, including assessment of PK interaction, will be presented. **Conclusions.** These preliminary phase 1 data suggest that weekly and twice-weekly MLN9708 in combination with MP is generally well tolerated with manageable toxicities. Preliminary anti-tumor activity in patients with previously untreated MM was observed.

0294

VANTAGE 095: FINAL RESULTS FROM A GLOBAL, SINGLE-ARM, PHASE 2B TRIAL OF VORINOSTAT IN COMBINATION WITH BORTEZOMIB IN SALVAGE MULTIPLE MYELOMA PATIENTS

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Background. Vorinostat (VOR) is an oral, multi-histone deacetylase inhibitor that affects pathways regulating cell proliferation and apoptosis in various tumor types. Phase 1/2 trials in MM have indicated clinical activity of VOR as both a single agent and in combination with bortezomib (BTZ) and immunomodulatory drugs (IMiDs). **Aims.** This global, open-label, single-arm, multicenter, phase 2b study was designed to determine efficacy and tolerability of oral VOR in combination with standard doses of BTZ in MM patients considered refractory to novel myeloma agents (eg, BTZ and IMiDs). **Methods.** Eligible patients were ≥18 years, received ≥2 prior regimens, and were BTZ-refractory (<25% response on therapy, progression during or <60 days after completion of therapy) and refractory to, intolerant of, or ineligible for IMiD-based therapy regimens. Patients received 21-day cycles of BTZ (1.3 mg/m^2 intravenously; days 1, 4, 8, 11) + oral VOR (400 mg/d; days 1-14); oral dexamethasone 20 mg on the day of and day after each dose of BTZ could be added to patients with progressive disease after 2 cycles or no change after 4 cycles. The primary endpoint was overall response rate (ORR; ≥ partial response). Responses and progression were determined according to the IMWG and the EBMT criteria for MM; efficacy data were confirmed by an independent adjudication committee. Patients were treated until disease progression, unacceptable toxicities, or withdrawal of consent, and monitored for safety. **Results.** A total of 143 patients were enrolled from 41 centers in 12 countries. In this heavily pretreated population, median duration of MM was 4.7 years, and 69% had received ≥4 prior regimens. Consistent with protocol inclusion criteria, all patients were considered refractory to prior BTZ. Of enrolled patients exposed to IMiDs, 99.3% had received ≥1 IMiD, 87% were considered refractory to ≥1 and 40% refractory to ≥2 different IMiDs, and 3% were ineligible for further IMiD-based therapy. ORR was 16.9%, with median duration of response of 170 days, corresponding to median progression-free survival of 3.13 months (95% CI: 2.40, 4.33); clinical benefit rate (≥ MR response) was 31.0%. Median overall survival (OS) was 11.23 months (95% CI: 8.47, 14.43), with a 2-year survival rate of 32%. Frequently reported adverse events were thrombocytopenia (69.7%), nausea (57.0%), diarrhea (53.5%), anemia (52.1%), and fatigue (48.6%); the overall safety profile was consistent with BTZ and VOR. Median exposure to study medication was 4 cycles, but in the absence of early disease progression patients tolerated the study treatment up to a mean of 6.2 cycles (range: 1-26). **Summary and Conclusions.** The combination of VOR + BTZ is active in MM patients refractory to novel treatment modalities and offers a new therapeutic option for this difficult-to-treat patient population. Additional high-risk and OS subgroup analyses will be presented at the meeting.

0295

A PHASE I/II TRIAL OF CYCLOPHOSPHAMIDE, CARFILZOMIB, THALIDOMIDE AND DEXAMETHASONE (CYCLONE) IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background. Carfilzomib is a proteasome inhibitor that irreversibly binds its target with a favorable toxicity profile that has shown significant activity in relapsed multiple myeloma (MM). Here we combined carfilzomib with a well known international regimen of cyclophosphamide-thalidomide-dexamethasone (CTD) in patients eligible for stem cell transplant (SCT). This would also reserve both bortezomib and lenalidomide for later in the course of the disease. **Methods.** We conducted a Phase I safety run in 6 patients with no DLT observed before expanding to Phase II. The phase II regimen is shown below. Treatment was for 4 cycles with expected SCT post induction. For the phase II portion of this trial, the primary endpoint is the proportion of patients who have ≥very good partial response to treatment. All patients received herpes zoster prophylaxis and ASA daily.Treatment schema was as follows on a 28 day cycle:Carlfilzomib 20 mg/m^2 cycle 1 (27 mg/m^2 ≥ cycle 2) IV Days 1,2,8,9,15,16Thalidomide 100 mg PO Days 1-28Cyclophosphamide 300 mg/m^2 PO Days 1,8,15Dexamethasone 40 mg PO Days 1,8,15,22**. Results.** Twenty seven patients were enrolled. Median age was 65 (range 27-74). Best overall response rate during 4 cycles of CYCLONE for evaluable patients (n=19) at phase II dosing is 100%: CR 32%, VGPR 47%, PR 21%. Grade 3 toxicity was reported in 52% of patients and 19% experienced grade 4 toxicity. Grade 3/4 toxicities occurring in >5% of patients included fatigue, neutropenia, lymphopenia, thromboembolism, and generalized muscle weakness. Two cases of pneumonia required hospitalization. Toxicities of any grade seen in >20% of patients included fatigue, constipation, lethargy, thrombocytopenia, somnolence, neutropenia, increased creatinine, malaise, sensory neuropathy, and depressed level of consciousness. Seven patients (26%) developed grade 1 sensory neuropathy; no higher grade or painful neuropathy was evident. All but one patient advancing to SCT successfully collected stem cells. One patient with t(4;14) disease who was not transplanted has progressed and one has died. **Conclusions.** The 4 drug CYCLONE regimen has remarkable efficacy (79% ≥VGPR, 100% ≥PR) and manageable toxicity in newly diagnosed patients with multiple myeloma. Especially notable was the low incidence of neuropathy and depth of response (CR 32%) after only 4 cycles. Given the relative lack of toxicity an extension of this regimen at higher doses of carfilzomib (20/45mg/m^2) has been initiated. Adding carlfilzomib to CTD as induction therapy is feasible and effective.

0296

INCORPORATION OF THALIDOMIDE-DEXAMETHASONE INTO DOUBLE AUTOTRANSPLANTATION FOR MULTIPLE MYELOMA: UPDATED ANALYSIS WITH A LONG-TERM FOLLOW-UP

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Background. Recent incorporation of novel agents into autologous stem-cell transplantation (ASCT) has enhanced the rate of high-quality responses, PFS and, albeit less frequently reported, OS. However, a limited number of studies assessing these outcomes with a long-term follow-up have been reported so far. **Aims.** To address this issue, we performed an intention-to treat analysis of 357 newly diagnosed MM patients who were followed for a median of 7 years from starting thalidomide-dexamethasone (TD) and subsequent double ASCT. **Methods.** Details of this multicenter phase 2 study were previously reported (Cavo et al, *J. Clin. Oncol* 2009). TD was given from the outset until the second ASCT. The cut-off date for the present analysis was December 31, 2011. **Results.** The rate of CR-nCR and ≥VGPR to induction therapy was 14.85% and 31.37%; the corresponding values after double ASCT were 36.69% and 60.78%. The median duration of CR was 53.1 months. Overall, median values of PFS and OS were 47.2 and 109.6 months, respectively. In addition to standard poor prognostic variables at baseline, both del(13q) (PFS: p=0.0001, OS: p=0.0002) and t(4;14)±del(17p) (PFS: p=0.0073, OS: p=0.0028) adversely affected clinical outcomes, the worst prognosis being associated with del(17p) [4-year PFS and 8-year OS estimates, 10% each vs 31% and 43%, respective-

ly, for patients who carried t(4;14) but lacked del(17p)]. Patients with del(13q) alone had a higher risk of relapse or progression (54% and 76% at 4 and 8 years) than those without any cytogenetic abnormality (p=0.0339), but similar PFS and OS curves due to the favourable effect of salvage therapies (median post-relapse OS: 30 vs 31 months, respectively). On multivariate analysis, failure to ever achieve at least VGPR, low Hb, high β2-m and t(4;14)±del(17p) were found to be independent variables predicting for poorer outcomes. In particular, a shorter OS was seen for patients ever lacking high-quality responses (HR: 0.35, 0.23-0.54, p<0.0001) and with t(4;14)±del(17p) (HR: 0.51, 0.33-0.79, p=0.0030). Overall, 20% of patients survived for more than 8 years; their median duration of CR was 77.5 months and the 8-year PFS estimate was 47%. The 8-year OS rate after relapse or progression was 72%, as compared with a median of 23 months for non long-term survivors (p<0.0001). On multivariate Cox and logistic regression analysis, attainment of high-quality responses predicted for, and was independently associated with, long-term survival (HR: 0.027, p=0.0050). **Conclusions.** With a median follow-up of 7 years, TD plus double ASCT extended PFS and OS up to the median values of 4 and 9 years, respectively. The corresponding figures with VAD plus double ASCT (Cavo et al, *J Clin Oncol* 2007), as updated at this meeting with a median follow-up of 5 years, were 3 and 6 years. Despite these advances, high-risk cytogenetics, particularly del(17p), retained their adverse influence on prognosis. Long-term (>8 years) OS probability was in the 20% range, with sustained CR in 24% of patients. Achievement of high-quality responses was the most important and independent variable affecting long-term OS. Prolonged survival after relapse was a contributing factor to long-term OS.

0297

CLINICAL FEATURES AND OUTCOME OF NEWLY DIAGNOSED, SYMPTOMATIC PATIENTS WITH MULTIPLE MYELOMA =80 YEARS OF AGE: AN ANALYSIS OF THE GREEK MYELOMA STUDY GROUP

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Background. Multiple myeloma (MM) is mainly a disease of the elderly and it has become common to treat patients of ≥80 years of age. However, octogenarians are often excluded from clinical trials due to comorbidities, very poor performance status or socioeconomic reasons. **Aims.** To describe the disease characteristics and identify prognostic factors in MM patients ≥80 years of age. **Methods.** We searched the database of the Greek Myeloma Study Group to identify patients with symptomatic myeloma who were ≥80 years of age at the time of initial therapy and who were treated in the novel agent era (between 1/1/2003 and 31/12/2010.) **Results.** Among 682 patients we identified 155 (23%) patients ≥80 years of age. Most octogenarians were males (57%) and had a poor ECOG performance status (PS ≥2) (60%); 70% had lytic bone disease, 53% anemia (Hb<10 mg/dl), 17% low platelet counts (<130x10^9/L), 11% elevated serum LDH (≥300 IU/L), while 59% had ISS-3, 34% ISS-2 and only 7% had ISS-1 MM (p=0.001). RI was common: 31% had serum creatinine ≥2 mg/dl but 63% had an eGFR <60 ml/min and 32% an eGFR <30 ml/min. When compared to patients <80 years of age, octogenarians had more often poor PS (p=0.001), anemia (p=0.006), elevated serum creatinine and low eGFR (p<0.001), low serum albumin (p<0.001) and elevated beta2-microglobulin (p<0.001). Upfront therapy with novel agents was given in 49% of octogenarians *vs*. 70% of patients <80 years (p<0.001). Response to first line therapy (≥PR) was lower in patients ≥80 years (58% *vs.* 78%; p<0.001). The median survival of patients ≥80 years was 22 months *vs.* 46 months for patients 66-79 years and 81 months for patients ≤65 years (p<0.001). Among octogenarians, 14% died ≤2 months from initiation of therapy *vs.* 3% for patients ≤80 years. In the univariate analysis, poor PS (16 months *vs.* 29 months, p<0.001) and elevated LDH ≥300 IU/L (9.4 *vs.* 27.5 months, p=0.016) were associated with poor survival in octogenarians. There were no significant differences in the characteristics of patients who were treated with novel agents or with conventional chemotherapy (CC). The median survival of octogenarians who were treated upfront with CC was 17 months *vs.* 26 months (p=0.3) for those who received upfront novel agents. Early death rates were similar. In an analysis, that excluded patients who died early (<2 months), response to first line therapy was associated with improved survival (29 *vs.* 16 months, p<0.001). In multivariate analysis, poor PS was the most important prognostic factor; upfront therapy with novel agents was independently associated with improved survival (HR: 0.64, 95%CI: 0.41-0.98, p=0.042), while male gender and ISS-3 MM were independently associated with poor survival. **Conclusions.** Patients ≥80 years of age with symptomatic myeloma often present with high risk features. Their median survival is <2 years and early mortality is high. Upfront use of novel agents is associated with significant survival benefit; however, reduced doses and aggressive supportive care are needed in order to reduce early mortality and to improve safety and patients' survival.

0298

SAFETY PROFILE AND MANAGEMENT IN MM-015 COMPARING LENALIDOMIDE-MELPHALAN-PREDNISONE FOLLOWED BY LENALIDOMIDE MAINTENANCE (MPR-R) WITH MP AND MPR IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM)

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Background. Lenalidomide is an IMiD® immunomodulatory agent with a dual mechanism of action comprised of tumoricidal and immunomodulatory effects. MM-015 is a pivotal, phase 3, randomized, double-blind, placebo-controlled trial comparing MPR-R with MP and MPR in transplant-ineligible NDMM patients. Interim results demonstrated significant median progression-free survival (PFS) improvements with MPR-R (31 months) vs. MP (13 months; *P*<.001) or MPR (14 months; *P*<.001). **Aims.** This analysis focused on the MM-015 safety profile and adverse event (AE) management; median follow-up was 41 months. **Methods.** Treatment details have been presented (Palumbo A, et al. *Blood*. 2011). AEs were graded according to the National Cancer Institute Common Terminology Criteria for AEs (V.3.0). For grade 4 hematologic and ≥ grade 3 non-hematologic AEs, treatment was withheld for the remainder of the cycle and restarted at a lower dose in subsequent cycles. Granulocyte colony-stimulating factor (G-CSF) or erythroid-stimulating agents were encouraged for grade 4 neutropenia or anemia. All patients received low-dose aspirin or other thromboprophylaxis where aspirin was contraindicated.

Table 1. Grade 3-4 hematologic and non-hematologic adverse events occurring in ≥ 10% of patients.

Treatment Arm	MPR-R		MPR		MP	
Age, years	65-75 (n = 114)	> 75 (n = 36)	65-75 (n = 116)	> 75 (n = 36)	65-75 (n = 116)	> 75 (n = 37)
Any hematologic, n (%)	90 (79)	33 (92)	86 (74)	32 (89)	58 (50)	18 (49)
Neutropenia	81 (71)	28 (78)	72 (62)	28 (78)	37 (32)	10 (27)
Thrombocytopenia	43 (38)	16 (44)	48 (41)	14 (39)	16 (14)	5 (14)
Anemia	27 (24)	12 (33)	27 (23)	16 (44)	22 (19)	4 (11)
Febrile neutropenia	5 (4)	5 (14)	3 (3)	1 (3)	0 (0)	0 (0)
Any non-hematologic, n (%)	64 (56)	23 (64)	59 (51)	26 (72)	50 (43)	18 (49)
Infections	10 (9)	7 (19)	14 (12)	8 (22)	10 (9)	5 (14)
Hypokalemia	3 (3)	4 (11)	2 (2)	4 (11)	0 (0)	1 (3)

Results. A total of 152, 153, and 154 patients were randomized to MPR-R, MPR, and MP, respectively. Grade 3-4 non-hematologic AEs occurring in ≥10% are listed (Table 1). During induction in the MPR-R, MPR, or MP arms, G-CSF was used in 100 (67%), 87 (57%), and 46 (30%) of patients. Only 1%, 4%, and 1%, respectively, discontinued any drug due to neutropenia. Regarding thrombocytopenia, no bleeding events were reported. Platelet transfusions were required by 51 (34%), 41 (27%), and 25 (16%) of MPR-R, MPR, and MP patients, respectively. Discontinuation of any drug for thrombocytopenia was infrequent: 3%, 5%, and 0%. With thromboprophylaxis, grade 3-4 deep-vein thrombosis (DVT) occurred in 3% (lenalidomide-treated) patients and 1% (MP) during induction. Discontinuation from induction due to AEs occurred in 16% (MPR-R), 14% (MPR), and 5% (MP) of patients. During lenalidomide maintenance, newly occurring or worsening grade 3-4 AEs were infrequent. Grade 4 neutropenia and thrombocytopenia were reported in 2/88 (2%) and 5/96 (6%), respectively. There were no reports of grade 3-4 febrile neutropenia or bleeding events. G-CSF was administered to 27 (31%) patients; 9 (10%) patients

received platelet transfusions. During maintenance, DVT was reported in 2 (2%) MPR-R and 1 (1%) MPR patients. Only 8% discontinued lenalidomide maintenance due to AEs. For induction and maintenance, discontinuation of any drug due to neutropenia was infrequent (2%, 4%, and 1%, for MPR-R, MPR, and MP, respectively). Similarly, only 3%, 5%, and 0% of patients discontinued any drug due to thrombocytopenia. Twelve (MPR-R), 10 (MPR), and 4 (MP) invasive second primary malignancies were reported, corresponding to low incidence rates per 100 patient-years of 3.04, 2.57, and 0.98, respectively. **Conclusions.** MPR induction AEs were manageable with dose reductions, G-CSF, and platelet transfusions, allowing the majority of patients aged 65-75 years to proceed to maintenance phase. Lenalidomide maintenance was generally well-tolerated in these patients with little evidence of cumulative toxicities, permitting continuous long-term therapy. In all patients, MPR-R treatment significantly extended median PFS vs. fixed-dose MP and MPR (*P*<.001 for both comparisons).

0299

LONG TERM SURVIVAL (> 10 YEARS) AFTER UP-FRONT SINGLE OR DOUBLE AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: RESULTS FROM A PROSPECTIVE CLINICAL TRIAL

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Background. Survival of patients with multiple myeloma (MM) has been extended with the introduction of autologous stem cell transplantation (ASCT). More recently, availability of highly effective novel agents has further improved patient outcomes. However, it is still the matter of debate whether a proportion of patients treated with ASCT can enjoy a long term survival (> 10 years) while sustaining prolonged high quality response. **Aims.** A large multicentre prospective clinical trial of single versus double ASCT was conducted in Italy from January 1996 to December 2001. To more carefully evaluate long-term clinical outcomes and to identify those variables which were related to long-term survival we performed a post-hoc analysis.*Methods:* A total of 321 patients were randomly assigned to receive either a single or double ASCT, as previously described (Cavo M et al, JCO 2007). Results were updated as of 30 December 2011 and compared with those previously reported. All the analyses were performed on an intention-to-treat basis. **Results.** After a median follow-up of 61 months for the entire treatment population and 120 months for survivors, both TTP and PFS remained significantly longer with tandem versus single ASCT (median 41 vs 25 months, P= 0.004 and 37 vs 25 months, P= 0.012, respectively), while OS was similar in the two groups (median 71 vs 67 months). Median OS after relapse or progression was 35 months in the entire population. 47% and 33% of the patients in the double and single ASCT group achieved a CR+nCR. Overall, in 24% and 11% of the patients CR+nCR was sustained for more than 5 and 10 years, respectively. In a multivariate Cox regression analysis, best response (CR+nCR) ever achieved was the most important variable significantly extending PFS (P= 0.003) and OS (P=0.050); random assignment to double ASCT was an additional variable predicting for prolonged PFS(P= 0.026). Overall, 23% of patients were alive over 10 years (long-term survivors). Median TTP and PFS of long-term survivors were 73 and 74 months, respectively, versus 26 and 25 months for the rest of the population (P= 0.0000). Median duration of CR+nCR was 70 months in the long-term survivors group in comparison with 21 months in the remaining patients (P= 0.0000). The 10-year estimate of OS after relapse or progression in this subgroup of patients was 58%, in comparison to a median value of 24 months for the control group (P= 0.0000). In a logistic regression analysis, best response ever achieved (CR+nCR) (OR: 1.8, 1.06-3.01, P= 0.03), haemoglobin level greater than 10 g/dL (OR: 2.0, 1.04-3.90, P= 0.04) and platelets count greater than 150.000/mmc (OR: 5.3, 1.59-17.66, P= 0.007) at diagnosis were independent variables predicting for long term survival. **Conclusions.** Twenty three per cent of the patients undergoing up-front single or double ASCT without novel agents were alive after 10 years from start of treatment, with 25% of patients remaining relapse free. Attainment of CR+nCR was the leading independent variable predicting for long-term OS. Prolonged survival after relapse was a contributing factor to long-term OS.

Non-Hodgkin lymphoma - Biology

0300

HIGH PD-1 EXPRESSION AND SUPPRESSED EFFECTOR CYTOKINE SIGNALING DISTINGUISH T CELLS INFILTRATING FOLLICULAR LYMPHOMA TUMORS FROM PERIPHERAL T CELLS

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Background. Defects in T cell function in cancer patients might influence their capacity to mount efficient anti-tumor responses to therapeutic vaccines. Previously, tumor infiltrating T cells (TILs) in patients with follicular lymphoma (FL) have been shown to have impaired immunologic synapse formation and T cell receptor signaling. **Aims.** 1) Identify effector cytokine signaling pathways specifically suppressed in FL TILs. 2) Determine whether T cell inhibitory receptors are differentially expressed on FL TILs. **Methods.** Single cell flow cytometry measurements of cytokine signaling were acquired for lymph node specimens from patients with FL (N=22), diffuse large B cell lymphoma (DLBCL; N=12) and mantle cell lymphoma (MCL; N=22). For comparison, blood samples from healthy donors (N=6) or from patients with chronic lymphocytic leukemia (CLL; N=14) were included. Phosphorylation of STATs were measured after activation with cytokines IL-4, IL-7, IL-10 or IL-21 in $CD3^+CD5^+$ T cells or in different T cell subsets using additional markers, including CD4, CD8, CD45RO, CD62L and PD-1. **Results.** We identified impaired IL-4-induced phosphorylation of STAT6 as well as impaired IL-10- and IL-21-induced phosphorylation of STAT3 in FL TILs as compared to DLBCL, MCL, CLL and healthy T cells. By combining phospho-protein specific flow cytometry with several T cell subset markers, we identified that CD4 T effector memory cells (T_{EM}; $CD62L^-CD45RO^+$), the major T cell subset present in malignant FL lymph nodes, was largely non-responsive to cytokines. This was not a general feature of $CD4T_{EM}$ cells since the majority of peripheral blood CD4 T_{EM} cells from FL patients could respond normally to cytokines. We observed high expression of the inhibitory receptor PD-1 in FL TILs, but not in autologous peripheral blood T cells in patients without leukemic disease. Importantly, detection of PD-1 expression in combination with p-STAT6 in FL LN samples stimulated with IL-4 revealed that $PD\text{-}1^{hi}$ FL TILs had lost their responsiveness, whereas $PD\text{-}1^-$ cells within the same sample had normal cytokine signaling responses. FL tumor cells were negative for PD-L1 and PD-L2, but tumor-infiltrating macrophages were $PD\text{-}L1^+$. Furthermore, disruption of the tumor microenvironment and *in vitro* culture for at least 24 hours was sufficient to restore cytokine signaling capacity in $PD\text{-}1^{hi}$ FL TILs, indicating that TILs *in vivo* seem to be getting the negative message through PD-1. **Conclusions.** FL TILs had impaired signaling downstream of key effector cytokines, including IL-4, IL-10, and IL-21. As the suppressed cytokine signaling was associated high expression of PD-1, our results suggest that therapies blocking PD-1 signaling activity might benefit FL patients.

0301

MOLECULAR CHARACTERIZATION OF IMMUNOGLOBULIN GENE REARRANGEMENTS IN DIFFUSE LARGE B CELL LYMPHOMA: A POSSIBLE ROLE FOR ANTIGEN SELECTION

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Background. Diffuse large B cell lymphoma (DLBCL) is an aggressive lymphoma that accounts for about 40% of adult B cell non-Hodgkin lymphomas (B-NHLs). However, ~25-30% of adult B-NHLs remain as DLBCL not otherwise specified (NOS). Immunostaining approaches allow these entities to be classified into GCB or non-GCB subtypes based on their presumed cell of origin. However, DLBCL pathogenesis remains partially unknown and the molecular features of immunoglobulin heavy chain (IGH) rearrangements may provide important information about the ontogeny and the role of antigen selection in DLBCL B cells. **Aims.** To study the IGH gene repertoire, mutation features,

and the stereotyped VH CDR3 patterns in order to contribute to a better understanding of the ontogeny and biology of DLBCL. **Patients and Methods.** A total of 165 patients diagnosed with DLBCL, NOS were included. Clonal IGH rearrangements were amplified according to the BIOMED-2 protocol and PCR products were directly sequenced. Germline IGH genes from complete IGHV-IGHD-IGHJ (V-D-J) and partial IGHD-IGHJ (D-J) rearrangements were identified using public IMGT/V-QUEST and BLAST databases, respectively. The canonical criteria for somatic hypermutation (SHM) (*Messmer et al. Blood 2004*) were analyzed in the global series as well as in the DLBCL subtypes and the most common IGHV genes. The criteria for stereotyped patterns (*Darzentas et al. Leukemia 2010*) in all complete VDJ sequences were carried out. **Results and Discussion.** Partial D-J rearrangements were present in 68/165 (41%), with 12% of them mutated (cut-off, 2%). Complete V-D-J rearrangements were identified in 130/165 (79%) patients. Most cases (89%) were highly mutated, but 12 sequences were truly unmutated or minimally mutated (100% or 97-99.9% germline identity, respectively). SHM was consistent with the canonical criteria, suggesting that the conventional machinery for mutations is used. A strong bias at the IGHV gene usage was observed, with IGHV4-34, IGHV3-23 and IGHV4-39 genes accounting for 1/3 of the cohort. The IGHV4-34 gene was overrepresented in our series (15.5% overall), appearing at a higher frequency than in normal B cells (3.9%, p<0.01). Finally, six cases were found to bear stereotyped sequences, three of them belonging to previously described subsets.Patients showing IGHV4-34 rearrangements shared several common features: 1) all but one were highly mutated; 2) all belonged to the non-GCB subgroup; 3) those in whom sequence could be obtained from the leader region conserved the specific FR1 motif (W7 and AVY 24-26), implying that these cells may retain the ability to bind to and be activated by superantigens, despite intense SHM activity. **Conclusions.** In DLBCL, IGHV gene usage is biased and bear stereotyped sequences, which imply an antigen-driven origin. The particular features in the sequence of the immunoglobulins suggest the existence of different biological subgroups within the non-GCB subtype. Their frequency and their possible clinical significance in DLBCL need to be analyzed in large independent series, which should be considered for future international collaborative efforts.

0302

CIRCULATING MICRORNAS AS PROGNOSTIC AND DISEASE RESPONSE BIOMARKERS IN PATIENTS WITH HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): A PROSPECTIVE AUSTRALASIAN LEUKAEMIA & LYMPHOMA GROUP STUDY.

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Background. MicroRNAs are small RNA molecules that play an important role in micromanaging gene expression. They have been strongly implicated in the pathogenesis of a variety of lymphomas. Their remarkable stability in both paraffin embedded formalin fixed (FFPE) tissue and peripheral blood make them excellent candidates as novel prognostic and disease response biomarkers with wide-spread applicability across lymphoma types. **Aims**. a) To identify microRNAs that are differentially expressed between a range of malignant and benign lymph nodes; b) To test whether highly expressed lymphoma-associated microRNAs had potential as circulating prognostic and disease response biomarkers in patients with high-risk DLBCL patients receiving R-CHOP chemo-immunotherapy. **Methods**. Agilent microRNA array (v2 and v3 chips) was performed on 23 DLBCL, 24 post-transplant lymphoma proliferative disorder (PTLD), 14 Hodgkin Lymphoma (HL) and 8 benign lymph nodes. A false discovery rate cut-off of 0.005 was applied. Real-time (RT-PCR) was used to confirm array findings. MicroRNAs consistently over-expressed in malignant nodes, regardless of histologic type, were then quantified in plasma using real-time RT-PCR, using a miR *Cel-39* spike in for normalisation. Plasma was obtained from patients enrolled into ALLG's prospective NHL21 study. This is a phase II clinical trial of high-risk DLBCL (IPI 2-5 and/or bulky disease), in which blood samples are taken at fixed time-points pre-therapy and post-cycle 4 of R-CHOP at time of interim PET/CT. 110 patients have been recruited to date. Informed consent was obtained for all trial participants. **Results.** miR-638 and miR-494 were consistently up-regulated across DLBCL, PTLD and HL nodes as compared to a benign lymph node signature. Levels of microRNAs 638 and 494 were markedly increased in the plasma of patients with high-risk DLBCL compared to normal controls (p<0.001 and p=0.003). Patients remaining PET/CT FDG-avid at the interim time-point had significantly higher pre-therapy plasma microRNAs (p=0.036) compared to patients who achieved a negative interim-PET/CT. Similarly at post-cycle 4, microRNAs were higher in those with a positive interim-PET/CT. **Summary and Conclusions.** A number of microRNAs are consistently differentially expressed between malignant and benign lymph node tissue. Circulating cell-free microRNAs in patients with high-risk DLBCL receiving R-CHOP show promise as prognostic and disease response biomarkers.

0303

QUANTITATIVE ASSESSMENT OF DAPK1 PROMOTER METHYLATION IN FOLLICULAR LYMPHOMA IS A PREDICTIVE PARAMETER OF DISEASE OUTCOME

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Backgroud. Transcriptional silencing of tumor suppressor genes, due to aberrant promoters' methylation, is a common epigenetic event in B-cell Lymphomas, including Follicular Lymphoma (FL). Using a qualitative analysis, we have previously reported that promoter hypermethylation of the pro-apoptotic death-associated kinase-1 (DAPK1) is a frequent epigenetic alteration in FL and correlates with an unfavorable outcome. **Aims**. The aim of our study was to study whether quantification of DAPK1 methylation levels could provide additional and more accurate prognostic information. **Methods.** We retrospectively studied 107 patients (49 males and 58 females), diagnosed at our Institution for FL in the period 2001-2011 of whom 91 patients were treated with immunochemotherapy (63 R-CHOP, 8 R-CVP, 11-R-FM, 9 other). DAPK1 promoter methylation, was determined on BM specimens by Methylight-PCR. As controls we included normal BM specimens obtained from 10 patients with ITP (Idiopathic Thrombocytopenic Purpura) and 10 PB samples from healthy volunteers. Quantitative results are expressed as percentage of methylation and the lower limit of detection of our assay was 0,1%.

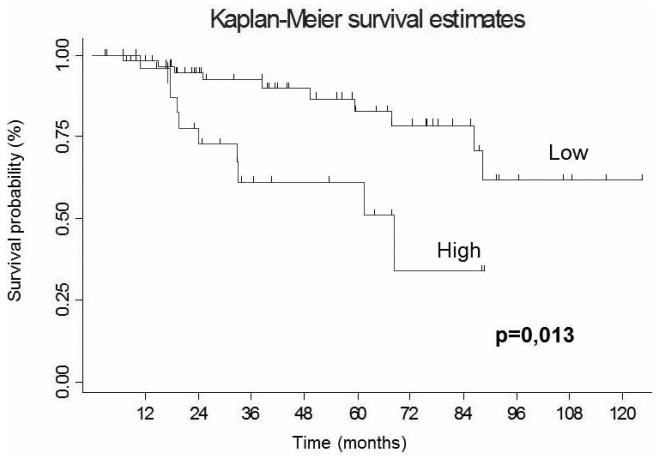

Figure 1. Unadjusted Kaplan-Meier plot of EFS according to DAPK1 methylation levels (high, >1.64%). p value of log-rank test is adjusted for treatment.

Results. In line with ourprevious data, we found DAPK1 promoter methylation in 73,8% of bone marrow samples of FL patients at diagnosis, while no methylation was found in our control group. The quantitative analysis demonstrated significantly higher methylation levels in patients with bone marrow infiltration (*p=0.0003*). Methylation levels were lower in patients with low risk disease according to both FLIPI and $FLIPI_2$ score (FLIPI 0-1 vs other: median 0,23% vs 0,53%; *p=0,0014*; $FLIPI_2$ 0 vs other: median 0,25% vs 1,12%; *p=0,025*). Levels of DAPK1 methylation were also associated to low histological grade (G1-G2 vs G3; medians: 0,41% vs 0,17%; *p= 0,004*). Bcl2/Igh rearrangement was present in 43.8% of our population, but no correlation was observed with DAPK1 methylation levels (bcl2/IgH positive vs bcl2/IgH negative; medians: 0.41% vs 0.24%; *p=0,35*). Interestingly, we found a significant higher level of methylation in patients not reaching Complete Remission (CR) after immunochemotherapy (*p=0,012*), suggesting a prognostic role for DAPK1 as epigenetic biomarker. Dividing in quartiles, methylation levels above the 75° percentile value of 1,64% predicted a significantly poorer outcome in terms of EFS (HR 3,2; 95% CI, 1,3-7,80; p=0,013 Figure 1; analysis adjusted for treat-

ment regimen). Including the FLIPI score into a multivariate analysis high levels of DAPK1 methylation (>1,64%) retained their impact on EFS as independent risk factor (HR: 2,8; 95% CI, 1,1-7,3; p=0,03). **Conclusions.** Our study indicates that the quantitative analysis of DAPK1 promoter methylation is a promising biomarker for Follicular Lymphoma, with prognostic impact on patients' outcome.

0304

LEVELS OF THE AUTOPHAGY ADAPTOR PROTEIN P62/SQSTM1 ARE NEGATIVELY CORRELATED WITH BCL-2 EXPRESSION IN HUMAN FOLLICULAR LYMPHOMA

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The autophagy adaptor protein p62/SQSTM1 delivers autophagy substrates, including itself, to autophagosomes for degradation. Accumulation of p62 within cells is therefore a marker of autophagy deficiency and elimination of p62 by autophagy has previously been proposed to suppress tumourigenesis. Since previous studies have suggested that the anti-apoptotic protein Bcl-2 can also inhibit autophagy, this study aimed to determine if Bcl-2 overexpression in human follicular lymphoma (FL) results in autophagy inhibition. We used p62 protein levels as a marker of autophagy and compared levels of p62 between FL and non-malignant reactive controls to ascertain the autophagy status of these cells.Using tissue microarrays (TMAs), p62 protein expression levels were determined in 156 reactive lymph node controls, 283 diagnostic FL and 154 matched relapse FL cores. By comparison to reactive lymph node controls, diagnostic and relapsed FL patients showed significantly decreased expression of p62 in germinal centres ($p < 0.001$). Bcl-2 positive FL patients (n=61) showed significantly reduced expression of p62 compared with Bcl-2 negative patients (n=25, $p < 0.001$). However, p62 expression levels neither predicted nor correlated with overall survival or time to relapse in FL patients.To examine this in more detail and to examine the cellular localisation of p62 in FL, levels of p62 and LC3-II, the generic autophagy marker, were examined in primary FL and reactive lymph node cells by Western blotting. Results showed an increased expression of LC3-II and decreased p62 expression in primary FL cells compared to reactive lymph node controls. The intracellular distribution of LC3 and p62 within FL and reactive lymph node cells was determined by immune-fluorescent microscopy. FL cells displayed higher numbers of LC3 punctate (LC3-II) and a diffuse distribution of p62, indicating active autophagy. However, LC3 staining of reactive control cells showed a diffuse, cytoplasmic pattern indicating non-autophagic LC3 (LC3-I) while greater numbers of larger p62-containing speckles were detected in these reactive cells. Together, these results demonstrate that FL patients have increased autophagy activity compared with reactive lymph node controls. We find no evidence to support an anti-autophagic role of Bcl-2 in primary FL cells.We therefore propose that overexpression of Bcl-2 in FL is negatively associated with p62 protein levels, suggesting that Bcl-2 does not inhibit autophagy in FL cells. Moreover, FL p62 protein levels are not associated with clinical outcome. Increased autophagy activity within FL may enhance resistance to chemotherapy.

0305

CKS1 IS REQUIRED FOR TUMOR CELL PROLIFERATION BUT NOT SUFFICIENT TO INDUCE HEMATOPOIETIC MALIGNANCIES

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Background. Cks1 is an activator of the SCFSkp2 ubiquitin ligase complex that targets the cell cycle inhibitor p27Kip1 for degradation. Loss of Cks1 results in p27Kip1 accumulation and decreased proliferation. Cks1 expression is elevated in various B cell malignancies including Burkitt lymphoma and multiple myeloma. We have previously shown that loss of Cks1 results in elevated p27Kip1 levels and delayed tumor development in a mouse model of Myc-induced B cell lymphoma. Surprisingly, loss of Skp2 in the same mouse model also resulted in elevated p27Kip1 levels but exhibited no impact on tumor onset. **Aims.** To test the hypothesis that Cks1 has yet unknown p27Kip1-independent functions in controlling cell cycle progression and to investigate the intrinsic ongogenic activity of Cks1 to induce hematopoietic disorders. **Methods.** Mouse embryonic fibroblasts of Cks1-/- and p27-/- genetic backgrounds were examined regarding growth, cell cycle distribution, Cyclin/CDK status and CDK activity. To challenge the oncogenic role of Cks1 we have overexpressed Cks1 in the hematopoietic system using a retroviral bone marrow transduction-transplantation system. **Results.** We identify a function of Cks1 in mammalian cell cycle regulation that is independent of p27Kip1 and other SCFSkp2 regulated CKIs. Specifically Cks1-/-; p27Kip1-/- mouse embryonic fibroblasts still show defects in the G1-S phase transition that are coupled with decreased Cdk2-associated kinase activity, and defects in proliferation that are associated with Cks1 loss. Furthermore, concomitant loss of Cks1 does not rescue the tumor suppressor function of p27Kip1 that is manifest in various organs of p27Kip1-/- mice. By contrast, defects in mitotic entry and premature senescence of Cks1-/- cells are p27Kip1 dependent. Regarding the oncogenic activity of Cks1 we show that despite potent ectopic overexpression, Cks1 was unable to promote B cell hyperproliferation, B cell lymphomagenesis, or to induce myeloid malignancies. This was at variance with c-Myc overexpression, which caused acute myeloid leukemia. **Conclusions.** Our studies reveal p27Kip1-independent functions of Cks1 in the regulation of the G1-S transition and an important role for Cks1 in tumor cell proliferation. Despite being overexpressed and associated with a poor prognosis in various hematological malignancies, sole Cks1 expression is insufficient to induce lymphoma or a myeloproliferative disease *in vivo*.

0306

HOST GENETIC POLYMORPHISMS IN TNF ALPHA, LYMPHOTOXIN ALPHA, AND IL-10 INFLUENCE RISK OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Polymorphic variants in genes encoding cytokines and involved in immune responses may alter the host's capability to fight cancer development. Tumor necrosis factor alfa (TNFa), lymphotoxin alfa (LTA), and interleukin-10 (IL-10) are key proteins involved in the regulation of immune responses and have potent effects on several types of immune cells. Polymorphisms in genes encoding these proteins have been studied with regard to how these polymorphisms may influence the risk of developing lymphoma and the response to lymphoma treatment in different populations. Altered risk for diffuse large B-cell lymphoma (DLBCL) have been found for specific variants of these genes, however, the numbers of included patients and controls are often small and the effect size often varied and conflicting. **Aims.** To investigate the impact of single nucleotide polymorphisms (SNPs) in selected cytokine genes on risk for developing DLBCL in a large population cohort. **Methods.** We genotyped 448 DLBCL patients (age 18-83 years) and 1056 blood donors as controls for polymorphisms in the genes *TNFa* (rs1800629, rs361525), *LTA* (rs909253), and *IL-10* (rs1800890, rs1800896), using PCR-amplification for the gene segments of interest and constant temperature capillary electrophoresis for analysis of the genotypes. **Results.** The *TNFa* rs1800629 A-allele was associated with increased risk of DLBCL (*TNFa* AG/AA combined vs. GG; OR 1.59 (1.27-2.00), p<0.001). The *LTA* G-allele, and *IL-10* rs1800896 A-allele were associated with decreased risk of DLBCL (*LTA* AG/GG vs. AA; OR 0.76 (0.60-0.95), p= 0.02, *IL-10* rs1800896 AT/AA vs. TT; OR 0.76 (0.59-0.98), p=0.04). For the *IL-10* SNPs, the control group was not in Hardy-Weinberg equilibrium, thus the interpretation of the results for the SNP *IL-10* rs1800896 should be made with caution. **Summary and Conclusions.** An increased susceptibility for DLBCL by polymorphisms in genes encoding TNFa, LTA and IL-10 was confirmed in a large cohort of patients and controls. The results support previous findings in other populations, and add evidence to the link between dysregulation of immune responses and risk of developing lymphoma.

Table 1.

Polymorphism	Genotype(s)	OR (95% CI)	p
TNFα	GG	ref.	
(rs1800629)	AG	1.54 (1.22-1.95)	<0.001
G>A	AA	2.03 (1.19-3.45)	0.009
	AG/AA	1.59 (1.27-2.00)	<0.001
LTA	AA	ref.	
(rs909253)	AG	0.71 (0.51-1.00)	0.05
A>G	GG	0.77 (0.61-0.98)	0.03
	AG/GG	0.76 (0.60-0.95)	0.02
IL10	TT	ref.	
(rs1800896)	AT	0.70 (0.54-0.92)	0.01
A>T	AA	0.89 (0.66-1.20)	0.45
	AT/AA	0.76 (0.59-0.98)	0.04

0307

A HUMAN LYMPHOMA TH17 CELL LINE MODEL

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Ehrentraut S, Schneider B, Nagel S, Quentmeier H, Kaufmann M, Meyer C, Kadin ME, Drexler HG, MacLeod RAFT-helper(Th)17 cells play key roles in autoimmune disease, notably psoriasis, Crohn's disease, juvenile diabetes, multiple sclerosis, and rheumatoid arthritis. Existing Th17 models are largely mouse based. Although the characteristics of human Th17 cells remain less well defined, it is apparent that human and mouse Th17 cells diverge both in their respective origins (CD161+ *vs* CD161- cells), and polarizing cytokines (IL-1β + IL-23 *vs* IL-6 and IL-1). Here we describe the characterization of a set of human cutaneous lymphoma cell lines deriving from a common T-cell clone to serve as a human *in vitro* model for those investigating Th17 biology. The donor patient initially presented with benign lymphomatoid papulosis followed later by Hodgkin lymphoma for which he received radiation treatment. Progressive skin disease returned, subsequently developing into a cutaneous T-cell lymphoma when the first cell line (MAC-1) was established. Two years later the patient developed a fatal anaplastic large cell lymphoma, from which two additional cell lines (MAC-2a and MAC-2b) were established. Microarray transcription analysis, validated by real time PCR, revealed that all three cell lines displayed conspicuous expression profiles highlighting genes expressed in T-effector cells. One terminal-phase cell line (MAC-2A) bore unmistakeable stigmata of Th17 cells, including conspicuous expression of IL-6, IL-17F, IL-22, ROR-a, STAT3 and TGFb. Prominent Th17 cytokine secretion was confirmed by ELISA and shown to be contingent upon JAK-STAT signalling. IL-17F expression was regulated by IL-6 signalling as reported in physiological conditions, since IL-6 receptor blockade effected by Tocilizumab treatment was accompanied by severe IL-17F downregulation. Conversely, when accompanied by treatment with the DNA methyltransferase inhibitor 5-azacytidine, exposure of chronic-phase MAC-1 cells to IL-6 stimulated IL-17F expression. Similar effects were produced by treatment with IL-2, a cytokine not hitherto implicated in Th17 development. Thus, MAC-1 cells display plasticity reminiscent of naïve T-cells and, together with MAC-2A cells present key aspects of T-effector cell development. Aryl hydrocarbon receptor (AHR) signaling reportedly supports IL-17 expression, and experiments performed on MAC cell lines confirmed the potential role of AHR signaling in IL-17 regulation. As well as providing an insight into human Th17 biology, our data support parallels between lymphoma development and T-cell polarization. We propose that analysis of MAC cells may reveal novel potential therapeutic targets and an *in vitro* model with which to test their druggability.

0308

INCIDENCE AND CHARACTERIZATION OF ADDITIONAL CHROMOSOMAL ABNORMALITIES IN MANTLE CELL LYMPHOMA (MCL) BY G-BANDING AND MOLECULAR CYTOGENETIC APPROACH.

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Background. Mantle cell lymphoma (MCL) is a distinct subtype of malignant lymphoma characterized by the translocation t(11;14)(q13;q32), resulting in overexpression of cyclin D1 and cell cycle dysregulation. Additional chromosomal abnormalities are present in 55~60% of patients and are associated with a potential role in the oncogenic process. **Aims.** We applied *G-banding*, FISH and spectral karyotype analysis (SKY) to better characterize the cytogenetic profile of secondary chromosomal abnormalities in 96 samples of blastoid MCL. **Methods.** Bone marrow and peripheral blood cells were used for *G-banding*, FISH and SKY analysis. Metaphase cells were obtained by using standard procedures and the subsequent cytogenetic analysis and interpretation of the data were made according to the ISCN (2009). FISH for detection of t(11;14)(q13;q32), including probes for del(6q), 8q23 rearrangements, del(13q)(*RB1* 13q14), and del(17p) *TP53* was performed on samples. Additionally we applied SKY analysis for those patients with complex karyotype (≥ 3 abnormalities). In order to confirm the molecular consequence of t(11;14)(q13;q32) and 8q23 rearrangements we evaluated the expression profile of cyclin D1 and *MYCC*, by real time PCR. **Results.** Secondary chromosomal abnormalities were present in 81.2% of patients. Structural abnormalities were more frequently observed than numerical (71,8% and 28,1%, respectively). Most of the structural rearrangements included balanced translocations. Abnormalities involving 6q (deletions and balanced translocations) represented 40.6% of the cases (n=39), by *G-banding* analysis. 6q abnormalities were also confirmed by FISH and those cases with complex karyotype we used SKY. On the other hand, deletions of 13q14 were observed in 45.8% of the patients (n=44), and FISH identified heterozygous deletions of *RB1* in all samples (n=44). Additionally, rearrangements involving 8q23 band represented 21.8% of the patients (n=21). However, among the profile of 8q23 rearrangements obtained, only ten patients presented Burkitt's like translocations [t(2;8)(p12;q24) and t(8;14)(q24;q32)]. *MYCC* hyperexpression was noted only in samples with t(2;8)(p12;q24) and t(8;14)(q24;q32). The remaining samples that displayed 8q23 rearrangements (n=11), we found normal levels of *MYCC*, compared with controls. Deletions of 17p were observed in 7 patients, by *G-banding* analysis. However, FISH for *TP53* identified loss of this gene in 14 samples investigated. SKY analysis and FISH helped to clarify the cytogenetic profile of samples with complex karyotype, including those, whose chromosome morphology was not suitable for *G-banding*. **Conclusions.** In conclusion, our findings demonstrate that the combination of *G-banding* and molecular cytogenetic approach, as for example FISH and SKY are important for detection and better characterization of secondary abnormalities in MCL. We noted that some specific abnormalities, such as 6q, 8q23 rearrangements and del(13q)(*RB1* 13q14) were more frequently observed in our samples than other studies involving secondary/additional chromosomal abnormalities in MCL, and reflect a consequence of genomic instability, disease progression and poor prognosis. This finding has important implications to predict outcome in MCL and should be considered in routine hematology laboratories.Financial support: FAPESP (Proc. 2007/52462-7 and 2011/01647-2).

0309

HAIRY CELL LEUKEMIA CELL LINES DISPLAY B-CELL RECEPTOR SIGNALS CHARACTERISTIC OF PRIMARY TUMOR CELLS BUT LACK THE SIGNATURE BRAF MUTATION TO REVEAL UNREPRESENTATIVE ORIGINS

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Background. In rare diseases such as Hairy cell leukemia (HCL), tumor-derived cell lines provide important models to study disease characteristics. A number of HCL derived cell lines have been generated and used successively to study key aspects of cellular function. Validation of these cell lines as representative of primary HCL disease remains incomplete. Recently the *BRAF* V600E mutation was established as a diagnostic molecular marker for typical HCL, identified in >99% of 350 HCL cases, mainly as a heterozygous mutation. **Aims.** To validate HCL cell lines as disease models of HCL by assessing B-cell receptor (BCR) function and the *BRAF* V600E mutation. **Methods.** We examined 6 HCL cell lines: Hair-M, HCLL-7876, EH, Eskol, HC-1 and HCLv-07. Surface marker and immunoglobulin (Ig) phenotype was performed by flow cytometry. Response to BCR stimulation with isotype specific goat $F(ab')_2$-anti-human Ig antibodes was assessed by measuring release of intracellular Ca^{2+} using fluo3-AM. ERK phosphorylation was assayed using the BD Phosflow method. Apoptosis was determined using ApoStat or Apo2.7 assays. For functional comparisons, tumor cells were selected in primary HCL by $CD19^+$, $CD11c^{HI}$, $CD103^+$ gating. *IGHV* genes were amplified from mRNA, cloned and sequenced. *BRAF* V600E mutation was analyzed in gDNA and mRNA by amplification and Sanger sequencing. **Results.** All cell lines expressed a single pre- or post- switched isotype, together with disease associated markers CD11c and CD103, although at a level of expression generally lower than in primary tumors, and were CD27 negative to mirror primary hairy cells. A single clonal somatically mutated *IGHV* gene was identified in 6/6 cell lines, again paralleling findings in the bulk of HCL tumors. BCR signals mediated Ca^{2+} flux and ERK phosphorylation in 4/4 cell lines, and in 2/2 lines were shown to trigger a marked apoptosis. These features recapitulated findings following BCR stimuli in primary HCL tumors, the data revealing that BCR function in cell lines is comparable to HCL disease. Strikingly however, we found that the *BRAF* V600E mutation was absent in all 6 cell lines, while readily confirmed in 4/4 primary s-HCL tumors. This reveals a clear divergence in the origin of the cell lines when compared with primary HCL. **Conclusions.** Although phenotype and BCR function in these cell lines reflects key features of primary malignant cells, the absence of the signature *BRAF* mutation clearly raises questions about their reliability as representative tools for the study of HCL disease. The findings furthermore illustrate the relevance and significance of molecular insights from next generation sequencing of tumor genomes when evaluating disease models.

0310

NOVEL EPIGENETIC BIOMARKERS FOR THE DETECTION AND PROGNOSIS OF NHL

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Background. Epigenetic changes are increasingly recognised as an important mechanism for altered gene expression in malignant cells. Altered DNA-methylation patterns may serve as biomarkers for cancer detection, assessment of prognosis, and prediction of response to therapy. **Aims.** Identify epigenetically regulated genes in B-cell lymphoma. **Methods.** We generated gene expression profiles from 11 B-cell lymphoma cell lines treated with 5-aza-2'deoxycytidine and trichostatin A and their normal counterparts. Further we analyzed the gene expression profiles of Non-Hodgkin-Lymphoma patients (n=638) and healthy controls (n=5). Epigenetically regulated candidate genes were subject to further analysis by methylation-specific PCR (MSP), bisulfite sequencing and quantitative MSP. **Results.** In the present study we identified epigenetically regulated genes by comparing gene expression profiles of 5-aza-2'deoxycytidine- and trichostatin A-treated cell lines to their untreated counterparts. Further we matched the upregulated genes after an epigenetic treatment with the gene expression profile of NHL patients and healthy controls. Only genes which were downregulated in patients and upregulated after epigenetic treatment in cell lines were subjected to further studies.We analyzed the methylation status of 30 candidate genes in lymphoma cell lines. Moreover, eight genes with a methylation frequency over 70% in cell lines were validated in NHL patients (n=56) and healthy controls, representing various stages of B-cell development. Preliminary results indicate that those genes are methylated in B-cell lymphoma patients and show no promoter methylation in healthy controls. We further analyzed the prognostic value of the candidate genes and found a correlation to survival. **Conclusions.** In conclusion, our results demonstrate that the analyzed genes might be suitable for early detection and monitoring of NHL.

0311

MOLECULAR CHARACTERIZATION OF THE 6Q23-27 REGION IN SEZARY SYNDROME

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Background. Sezary's syndrome (SS) is a rare form of cutaneous T-cell lymphoma characterized by erythroderma and the presence of Sezary cells (CD3+, CD4+, CD8-) in skin, lymph nodes, and peripheral blood. Chromosomal instability is characteristic of this lymphoma and related to bad prognosis, but no specific abnormalities that may be directly involved in the development of the disease have been found yet. Over the past few decades cytogenetic and molecular cytogenetic findings revealed many genetic alterations in patients with SS, including recurrent losses in the 6q region, which is frequently altered in different cancer types and might carry genes important in oncogenesis. Detailed molecular characterization of DNA changes has the potential to reveal genes being deregulated and possible gene fusions. **Aims.** The purpose of this project was to analyze the 6q23-27 region at the molecular level in 12 Sezary syndrome patients and Sezary syndrome cell line (SeAx). **Methods.** High resolution Fine-Tiling Comparative Genomic Hybridization (FT-CGH) was performed for the 6q23-27 region and deletions were detected in 6 patients and SeAx cell line. Previous studies had shown that copy number changes detected by the FT-CGH, might be associated with small structural aberrations, like inversions or translocations, at the breakpoint. In order to sequence each breakpoint and identify possible rearrangements, samples with deletions were further analyzed by Ligation-Mediated PCR (LM-PCR). Moreover, in two samples paired-end next-generation sequencing was performed on the HiSeq2000 Illumina platform. **Results.** Using those techniques 37 breakpoints were characterized (28 in SS patients and 9 in SeAx), and 21 new rearrangements were identified, including 7 simple deletions, 8 inversions, 3 translocations (with chromosomes 3, 10 and 12) and 3 transpositions. 14 genes were disrupted as a result of those rearrangements, and two of them: oncogene *MYB* and *IL22RA2*, were affected more than once. **Conclusions.** In summary, the combination of FT-CGH and LM-PCR, as well as the high-throughput paired-end sequencing approach revealed numerous different rearrangements, including inversions and translocations, associated with the deletions in the 6q23-27 region in Sezary syndrome. Further studies are needed to unravel the effect of the identified alterations on the biology of SS cells and their possible role in the malignant transformation.

0312

ONO-WG-307, A POTENT INHIBITOR OF BRUTON'S TYROSINE KINASE (BTK), INDUCES TUMOUR SUPPRESSION IN ABC-DLBCL (TMD-8) XENOGRAFT MODEL

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Background. Bruton's tyrosine kinase (Btk) is a key regulator of B-cell receptors (BCR), which play a central role in signal transduction pathways regulating survival, activation, proliferation, and differentiation of B-lineage lymphoid cells. Abberant BCR signaling is implicated in the survival of malignant B-cells and recent studies indicate that targeting Btk may be effective in the treatment of B-cell lymphoma. **Aims.** ONO-WG-307 is a highly potent and selective Btk inhibitor with an IC50 in the sub-nmol/L range. The activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL) is associated with poor prognosis and new therapies, preferably chemo-sparing therapies, or as add-on to existing treatment regimens, are required to help treat patients with ABC-DLBCL. Therefore, Btk constitutes an interesting therapeutic target, thus the activity of ONO-WG-307 was evaluated in an ABC-DLBCL xenograft model. **Methods**. TMD-8 tumour cells were implanted subcutaneously into female SCID mice and ONO-WG-307 was administered orally, twice a day (BID) at doses of 1 mg/kg, 3 mg/kg and 10 mg/kg. Treatment with ONO-WG-307 was initiated when the mean tumour volumes reached 100-200mm^3. In order to investigate the activity of ONO-WG-307 in more established tumours, ONO-WG-307 was administered at a dose of 10 mg/kg BID in animals with mean tumour volumes of 300-400 and 500-600 mm^3. Animals were euthanized when the tumours reached a maximum volume of 2,000 mm^3 or after a maximum period of 2 months. **Results.** Treatment with ONO-WG-307 resulted in a dose-dependent inhibition of tumour growth in the TMD-8 xenograft model. Furthermore, parallel analysis of a pharmacodynamic marker using peripheral blood mononuclear cells (PBMCs), measuring phosphorylated Btk (P-Btk), further supported that Btk was inhibited and the level of P-Btk inhibition was correlated with the observed decreased tumour volumes in the TMD-8 model. **Conclusions.** ONO-WG-307 is a highly potent and selective oral Btk inhibitor with evidence of efficacy in the ABC-DLBCL TMD-8 xenograft model, with Btk inhibition further supported by using a PD marker from PBMCs. Given the need to treat and overcome disease resistance especially in ABC-DLBCL, the use of a Btk inhibitor is a novel, mechanistic approach to treating B-cell malignancies and these results warrant further development of ONO-WG-307 in clinical studies. Additional combination studies are underway using the TMD-8 xenograft model testing ONO-WG-307 in combination with various agents.

0313

LYTIC PHASE REPLICATION OF EPSTEIN-BARR VIRUS IN REPLICATION COMPETENT LEUKEMIA/LYMPHOMA CELL LINES

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Epstein-Barr virus (EBV) is widespread in human populations and associated with diverse malignant tumors, including Burkitt, Hodgkin and T/NK-cell lymphoma, nasopharyngeal and gastric carcinoma. EBV is also widely used for the *in vitro* immortalization of human B-cells to establish B-lymphoblastoid cell lines (B-LCL). The gammaherpesviruses persist latently in the form of covalently closed circular episomes in B-cells, expressing various latent phase genes. In a number of B-LCL or EBV-infected leukemia/lymphoma cell lines a fraction of the cells enter the lytic phase of infection, producing active viruses spontaneously or after induction with particular stimuli. The mechanisms of virus reproduction, especially the formation of the packaged linear virion DNA from the circular episomes, are not well understood. The linear genomes are characterized by repetitive terminal sequences at the 5´- and 3´-end of the EBV genome. Until now, a concatemeric replication of the episomal DNA was postulated according to the appearance of terminal repeat (TR) regions of variable length

reflecting the numbers of repetitive exons. To determine which cell lines are able to enter the lytic phase of reproduction, we applied different methods to differentiate cell lines restricted to the latent phase from those containing cells in the lytic phase. In contrast to latent phase cell lines all cell lines with lytic cells showed a laddering in Southern blots, resulting from viral genomes with different numbers of TRs. Hybridizing the blots with probes from the 5´- or 3´-prime end of the EBV genome revealed free DNA ends with TRs as well as fused TRs. However, the fused TRs also showed a marked laddering, whereas the episomal genomes of the latent cells displayed a single band of fused TRs only. Additionally, applying pulsed-field gel electrophoresis to separate large DNA fragments revealed no linear DNA with a number of consecutive ca. 170 kb EBV genome units. Comparing the episomes of various lytic phase cell lines, most of them display different numbers of TRs which is, however, constant within a given cell line. Cloning and sequencing the TR region of various fused genome ends of the phorbolester- and sodium butyrate-stimulated B95-8 cell line demonstrated TR structures identical to the episomal TR structure. This indicates that always complete TR units are either "lost"during replication by sequence specific events (for instance looping out) or are actively excised during or after the replication process. However, the results clearly disprove the concatemeric replication of EBV. The elucidation of the exact replication mechanism might uncover an Achilles heel of EBV or even all herpesviruses which might be used to eliminate these viruses and to preclude many hematopoietic and other malignant tumor.

0314

WITHDRAWN

0315

SOLUBLE INTERLEUKIN-2 RECEPTOR LEVEL PREDICTS SURVIVAL IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Background. Recent data show that host antitumor immunity is actively suppressed by cancer cells in many ways. The key mediator of immune tolerance is IL-2 stimulating differentiation of host CD4+ cells into suppressive CD4+CD25+ T-regulatory cells. Soluble IL-2 receptor is generated exclusively by the proteolytic cleavage of membrane IL-2R. Soluble complex IL2R-IL-2 leads to prolonged persistence of IL-2 signaling. **Aims.** To analyze the prognostic impact of pretreatment sIL-2R levels on FL patients' survival in the rituximab era.Methods: We studied 88 patients with newly diagnosed FL (median age 57 years). The FLIPI risk groups were: high 43%, intermediate 27%, low 30%. Most patients (77%) had advanced-stage disease; bulk >7cm was present in 49%. Almost half of them had elevated B2M level (>3mg/L, 45%). The mean (median) sIL-2R level at diagnosis was 188.5±174.3 kU/L (132.6 kU/L); elevated sIL-2R ≥115 was detected in 48/88 patients (55%). The GELF criteria were met by 79 (90%) patients. Treatment was applied with respect to their disease: CHOP/CHOP-like regimen in low-intermediate-risk FL (n=40, 50%) and more intensive protocol (Promace-Cyta-BOM/adequate intensity protocol) in intermediate-risk patients with additional unfavorable risk factors (high B2M, s-TK or bulky; n=10, 13%). Nineteen (24%) patients were treated with upfront ASCT (conditioning BEAM 200) due to very high-risk disease. Rituximab was added to frontline therapy in 66% and maintenance immunotherapy with rituximab (or interferon) was applied in 31% (39%) of cases. Patients with high sIL-2R share more unfavorable prognostic features than low sIL-2R group: H-FLIPI (60% vs 23%, p<0.001), bulky disease >7cm (70% vs 28%, p<0.001), advanced clinical stage (92% vs 60%, p<0.001), elevated B2M (60% vs 28%, p=0.002). No differences were observed between both groups in terms of age distribution, rituximab or maintenance application. Patients with high sIL2R needed more intensive therapy approaches: 25/29 (86%) patients treated with intensive protocol or ASCT were from high sIL2R group. By contrast, all 9 patients with no need for therapy had low sIL2R (p=0.01). **Results.** Complete remission rate was 83% and was comparable in both groups (81% vs 85%, p=0.67). After a median follow-up of 67 months (5.6 yrs), only 25/48 (52%) high-sIL-2R patients are alive in the 1st CR, compared to 29/40 (73%) in low sIL-2R group. Five-year progression-free survival was 54% (95% CI 0.39-0.68) in high-sIL-2R group, compared to 66% (95%CI 0.49-0.83, p=0.05) in high-sIL-2R group. Overall survival was not significantly different between both groups (p=0.14) despite more than twice as many events observed in high-sIL-2R group (13 vs 5 deaths). **Conclusions.** Soluble IL-2R plays an important biological role in tumor immunosurveillance. This fact reflects the presence of advanced disease features such as bulky disease, high B2M and higher FLIPI scores. Despite application of more intensive treatment approaches, the outcome of patients with high sIL-2R is less favorable. This parameter should be considered an adverse negative prognostic factor.Supported by grants from Czech Ministry of Education (MSM 6198959205) and Faculty of Medicine and Dentistry, Palacky University Olomouc (LF-2011-006).

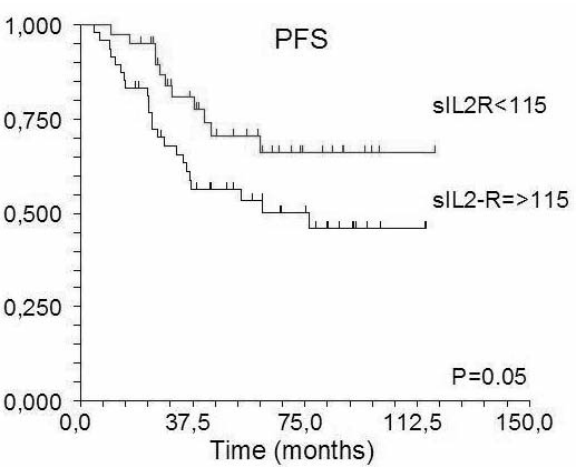

Figure 1.

0316

SERUM BETA2-MICROGLOBULIN IS AN INDEPENDENT STRONG PREDICTOR OF OS IN A POPULATION OF DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATIENTS TREATED WITH R-CHOP

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Background. Stratification of diffuse large B-cell lymphomas (DLBCL) patients (pts) according to clinico-pathological features at diagnosis represents a major challenge. **Aims and Methods.** We retrospectively analyzed the clinical variables at diagnosis and outcome of a population of consecutive pts with confirmed diagnosis of DLBCL from the databases of the Oncology Institute of Southern Switzerland in Bellinzona (Switzerland) and the Division of Hematology of the Amedeo Avogadro University of Eastern Piedmont, Novara (Italy).Results. Among 2,426 non Hodgkin's lymphoma pts treated since 1980 to 2011, 813 cases of DLBCL were selected and, among these, 364 patients (187 male, 177 female), treated with combination of rituximab and CHOP (or CHOP-like) chemotherapy, were identified. In this pts subset, median age at diagnosis was 66 years (18-89);209 pts(57%) had stage III-IV, extranodal disease was reported in147 pts(40%), serum LDH was elevated in190 pts(54% of 352 tested pts),193 pts(60% of total321 pts) had elevated serum beta2-microglobulin; 161 pts (45% of the 360 cases in whom the data was available) had intermediate-high/high International Prognostic Index (IPI) risk. The distribution of351 ptsevaluable according to the revised-IPI (R-IPI) was the following:36 pts(10%) had low risk,159 pts(46%) had intermediate risk,156 pts(45%) had high risk. After median follow-up of 3.4 years, median OS was not reached yet, projected 3-yrs OS was 75% (95%CI:70-90), 5-yrs OS was 66% (95%CI:59-72) and 10-yrs OS was 53% (95%CI:37-66). The serum beta2-microglobulin level significantly predicted OS (P<0.0001. Figure 1). The projected 5-years OS was 83% (95%CI:73-90) in pts with low beta2-microglobulin at diagnosis and 55% (95%CI:45-64) in cases with high levels. The IPI and R-IPI prognostic models confirmed their capacity to predict the outcome in this pts population (P<0.0001 for both models). Beta2-microglobulin retained its

prognostic significance at multivariate analysis controlling for the single variables included in the prognostic models (IPI and R-IPI) and for each single model as a whole [HR: 2.39 (95%CI: 1.33-4.32) for the comparison with R-IPI]. **Conclusions.** In an era in which more and more genomic markers are suggested the prediction of prognosis of DLBCL pts according to clinical variables at diagnosis still has a major role in everyday clinical practice. In our series, the serum beta2-microglobulin was an important and independent prognostic marker of OS in R-CHOP treated pts in addition to R-IPI score. Beta2-microglobulin remains a reliable tool to be included in new prognostic indexes.

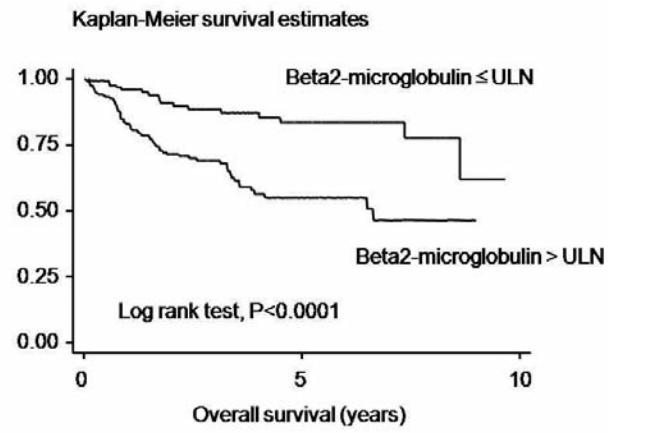

Figure 1.

0317

CLINICAL SIGNIFICANCE OF SUPPRESSOR OF CYTOKINES SIGNALLING-3 EXPRESSION FROM PATIENTS WITH NON HODGKIN LYMPHOMA UNDER CHEMOTHERAPY

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Introduction. To date, little is known about blood immune marker changes that may be related to the development of Non Hodgkin Lymphoma (NHL) and treatment response. And there are few circulating biological markers that could be useful in follow up of the patients. Objective: To study, by means of reverse-transcription polymerase chain reaction (RT-PCR), the expression of suppressor of cytokine signalling-3(SOCS-3) gene at the mRNA level in peripheral mononuclear cells from patients with NHL and to correlate with clinical pathological features and response to treatment. **Patients and Methods.** Thirty patients with NHL and 20 healthy controls were enrolled in the study. The SOCS-3 mRNA level in peripheral blood mononuclear cells (PBMCs) was detected by semi-quantitative real-time polymerase chain reaction. Quantification of cytokines such as interleukin 6 and tumor necrosis factor alpha (IL- 6 & TNF-α) were performed using sandwich enzyme-linked immunosorbent assays (ELISA). Main **Results.** Increased expression of SOCS-3 mRNA in peripheral blood plus increased serum levels of IL- 6 and TNF alpha from NHL cases with no complete remission after therapy. Higher levels of expression of SOCS-3 are associated with advanced disease, bone marrow involvement, extranodal involvement, poor performance status, B cell symptoms and high serum lactate dehydrogenase level which are evaluated by international prognostic index (IPI). Complete responses occur in 60% of patients with normal expression of SOCS-3 gene. Increased expression of SOCS-3 is common in diffuse large B cell lymphoma, CLL/small lymphocytic B cell lymphoma and follicular lymphoma. **Conclusions.** Elevation of mRNA expression of SOCS-3 correlates with advanced disease and poor response to treatment. Peripheral SOCS-3 expression might be used to monitor response during treatment. **Keywords.** Suppressor of cytokine signaling-3 expression; IL-6, TNF-α and non-Hodgkin's lymphoma

0318

ABSOLUTE MONOCYTE COUNT IS ASSOCIATED WITH ADVERSE PROGNOSIS IN DIFFUSE LARGE B-CELL LYMPHOMA: A VALIDATION STUDY IN A COHORT OF 219 PATIENTS FROM TWO CENTERS.

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Background. There is an emerging and growing interest regarding the role of monocytes and monocytic-derived cells in lymphoproliferative disorders. Recently it has been shown that high peripheral blood monocyte count is an independent prognostic value associated with poor prognosis and decreased overall survival in patients with diffuse large B-cell lymphoma (DLBCL). **Aims.** To evaluate the prognostic significance of monocytosis in a large cohort of newly diagnosed patients with DLBCL, to verify possible correlations with other well established prognostic factors, and to determine whether the administration of rituximab abrogates the adverse effect of elevated monocyte counts. **Material and Methods.** We reviewed clinical and laboratory data of consecutive untreated patients with DLBCL followed and treated at two medical centers in Israel between1993-2010. Monocytosis was defined as a count of > 800 cells/ mm^3 based on the count before starting treatment .This parameter was also evaluated for possible correlations with other prognostic and clinical factors including age, gender, B symptoms, stage, extra nodal involvement, serum LDH, bone marrow involvement, and the IPI score. All patients were treated with CHOP or rituximab (R)-CHOP regimens. A bivariate analysis for monocytosis and rituximab vs non-rituximab based therapy in relation to overall survival was performed.We conducted Kruskall-Wallis and Chi-Square Tests when appropriate. Log-rank tests was performed for survival analysis and a Cox proportional hazards regression model was used to determine the significance of the prognostic factors in a multivariate analysis. **Results.** 219 patients from two different medical centers in the north of Israel were evaluated. The median age at diagnosis was 59 years (range 18-96 yrs). The median follow-up time after diagnosis was 67.5 months (range, 1 to 332 months). In order to evaluate the relevance of monocytosis we compared the two categories (> 800 and < 800 cells/ mm^3) with other prognostic parameters. Monocyte counts above 800 cells /mm3 were associated with older age (66 vs 57 years) p=0.018, presence of B symptoms (40 % vs 23%) (p= 0.025), elevated LDH levels (67.4% vs 50%) (p=0.043), and high IPI score (26.6% vs 9.8%) (p=0.029). There was no correlation between monocyte count, gender and lymphopenia (lymphocyte count<1000 mm^3).The 5 years OS of the entire cohort was 78.7% and patients with monocytes above 800 / mm^3 had a 5 years OS of 63%, compared to 83% in those with monocytes<800/ mm^3 (p<0.01). Fifty seven patients (26.6%) were treated with CHOP alone, while 157 (73.4%) received R-CHOP. The addition of rituximab to CHOP combination chemotherapy did not abolish the adverse effect of monocytosis (p=0.73). **Conclusions.** This validation study in a larger patient cohort confirms the results reported in an earlier study and shows that monocytosis, a simple and readily available prognostic parameter, can easily be applied routinely to evaluate newly diagnosed patients with DLBCL and identify those with an higher risk for poor survival. The addition of rituximab, an agent with a well-recognized immuno-modulatory effect on the microenvironment, including monocytes , did not abrogate the negative adverse effect of monocytosis.

0319

TELOMERE LOSS IS AN EARLY AND PERSISTENT ABNORMALITY IN LEUKOCYTES FROM PATIENTS TREATED WITH CONVENTIONAL CHEMOTHERAPY BUT NOT FROM THOSE EXPOSED TO RITUXIMAB ALONE

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Background. Patients with lymphoproliferative disease very often require treatments with chemotherapy. Several recent reports suggest that the exposure of leukocytes to chemotherapeutic drugs, may induce premature cell ageing. A good indicator of cell replication history is the length of telomeres. Indeed, telomere shortening and/or telomere dysfunction have been documented in patients receiving chemotherapy. The loss of telomere sequences has been linked to the increased risk of developing secondary malignancy in subjects previously treated with chemotherapy. However, the cell type involved and the time course for the onset of chemotherapy-induced telomere shortening remain to be elucidated. In the present study changes in telomere length (TL) before and after cytotoxic drug exposure were evaluated. **Aims.** Main aims of the study were: i. to verify whether TL shortening is a phenomenon induced by extensive chemotherapy treatments or it may occur even after minimal drug exposures; ii. to identify possible cell populations that are particularly susceptible to drug-induced telomere; iii. to define whether TL shortening following chemotherapy is reversible or permanent; iv. to investigate the different effect of chemoimmuno- or immunotherapy alone. **Methods.** Peripheral blood (PB) cells were obtained from 15 lymphoma patients undergoing chemoimmunotherapy (8 R-CHOP, 3 ABVD, 2 R-Bendamustine, 1 R-MINE, 1 R-OXDHA) and 5 patients with primary immune thrombocytopenia (PTI) treated with the anti-CD20 Rituximab (R). Median age of patients was 45 years. All but two lymphoma patients were at their first treatment line. TL was assessed on granulocyte (GN), mononuclear cell (MNC) and on total leucocytes (total PB) before and after each chemotherapy course. In 9 lymphoma patients and in all PTI, TL was assessed also at long term since last therapy. TL was evaluated by southern-blot analysis. **Results.** A marked reduction in TL was detected in 14/15 (93.3%) patients undergoing conventional chemotherapy in all PB cells investigated. As shown in Figure 1, a marked TL loss following chemotherapy compared to pre-treatment values was observed in granulocyte (p=0.0001), although a TL reduction was detectable also in MNC (p=0.003) and total PB (p=0.0009). In most patients TL shortening was detectable already after the first (9 pts) or the second (3 pts) chemotherapy course. In addition, TL shortening remained virtually unchanged up to 6 months since the last therapy in all 9 patients evaluated at long-term. Among different chemotherapy schemes evaluated, TL shortening was less pronounced in R-CHOP treated patients compared to patients receiving the other schemes, although the difference was not statistically significant. No difference in TL was detected before and after drug exposure in the five patients receiving R monotherapy, even in patients followed up to 10 months since last R infusion (Figure 1). **Conclusions.** Results indicate that telomere shortening: i. can be detected early following chemotherapy exposure and is persistently detectable for several months since chemotherapy; ii. can be most easily detectable in granulocytes; iii. is less evident in RCHOP treated patients compared to other therapy; iv. is not observed in patients affected by PTI treated with Rituximab.

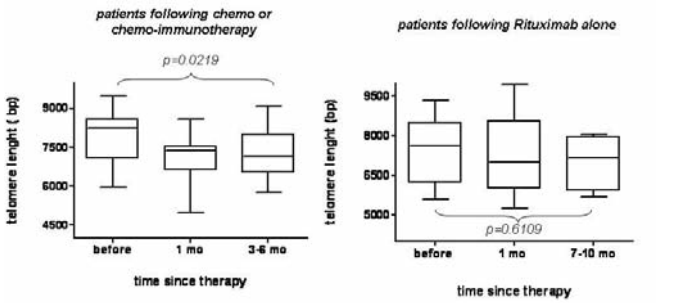

Figure 1. Changes of telomere in patients following chemo or chemo-immunotherapy and in patients treated with Rituximab alone.

0320

THE HIGHEST PROGNOSTIC IMPACT OF LDH AMONG INTERNATIONAL PROGNOSTIC INDICES IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN THE RITUXIMAB ERA

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Background. The International Prognostic Index (IPI) has been the primary clinical tool used to predict outcome for patients with diffuse large B-cell lymphoma (DLBCL). Although the IPI is considered as the standard prognostication system currently used, heterogeneity in prognosis still exists among patients with the same IPI score or risk group. Furthermore, the relative significance of each clinical factor of the IPI in same prognostic group has not been previously evaluated. **Aims.** We aimed to investigate the prevalence and relative prognostic impact of each risk factor of IPI in DLBCL patients with the same IPI scores. **Methods.** We retrospectively analyzed 387 patients treated with the standard R-CHOP as front-line therapy in a single center between February 2002 and February 2010. Patients with each IPI score were assigned to subgroups according to the combination of presenting risk factors comprising IPI: A (age ≥60 years), L (elevated serum LDH level), P (ECOG performance ≥2), S (Ann-Arbor stage ≥3), and E (extranodal sites ≥2). Then we investigated the frequency of the subgroups and compared their efficacy outcomes including complete response (CR) rate, 5-year event-free survival (EFS) rate, and 5-year overall survival (OS) rate. **Results.** The IPI remained predictive of EFS (p <0.01) and OS (p <0.01) in this study cohort. And the frequency of each IPI score was: 0 - 23.3% (n=90), 1 - 23.0% (n=89), 2 - 19.4% (n=75), 3 - 19.6% (n=76), 4 - 12.1% (n=47), 5 - 2.6% (n=10). Elevated LDH (n=209, 54.0%) was the most common risk factor in total, followed by S (n=207, 53.5%), A (n=142, 36.7%), E (n=111, 28.7%) and P (n=36, 9.3%). And in each IPI score of 1 to 4, A (n=36, 9.3%), LS (n=36, 48.0%), LSE (n=36, 47.4%), and ALSE (n=30, 63.0%) were the most predominant subgroups. Patients with elevated LDH tended to have lower CR rate, inferior EFS and/or OS irrespective of the IPI scores. Especially, patients with high LDH among those with IPI score of 2 had lower CR rate (73.1% vs. 95.2%, *p*=0.024), 5-year EFS (57% vs. 87%, *p*=0.014) and 5-year OS rates (58% vs. 82%, *p*=0.027) with statistical significance. **Conclusions.** Heterogeneity in prognosis among patients with the same IPI score still exists. Among the risk factors of IPI, LDH seems to be the most prevalent and potentially most significant prognostic factor for patients with DLBCL, which requires further validation though.

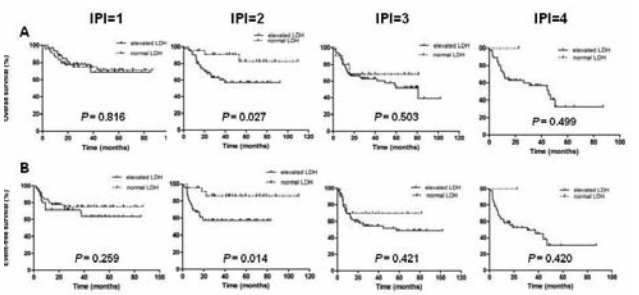

Figure 1. Overall survival (A) and event-free survival (B) of IPI score 1 to 4 by LDH level.

Myelodysplastic syndromes - Biology

0321

PROSPECTIVE PHASE II STUDY ON LOW-DOSE 5-AZACYTIDINE FOR TREATMENT OF SYMPTOMATIC PATIENTS WITH LOW/INT-1 RISK MYELODISPLASIA

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Background. Aberrant DNA-methylation in the CpG sites of several tumor suppressor genes is considered the dominant pathogenetic mechanism in MDSs and the main cause of progression to AML. The use of hypomethylating agents significantly modified the therapeutic approach to MDS patients, primarily in higher-risk MDS patients. In lower-risk MDSs the use of 5-AZA hypomethylating agent is less understood. **Aims.** We prospectively evaluated the efficacy and safety of 5-AZA low-dose in Low or Int-1 risk MDS patients who were symptomatic and/or unresponsive to previous treatments. Furthermore, we studied the genetic profile by single nucleotide polymorphism (SNP) arrays and the molecular effects of 5-AZA on PI-PLCbeta1 promoter methylation, in order to identify these biological factors possibly correlated with the response to 5-AZA. **Methods.** 5-AZA was administered at a dose of 75 mg/mq/daily s.c for 5 consecutive days every 28 days for a total of 8 cycles. Final response was checked at the end of the 8th course. SNP microarray analysis was performed from mononuclear cells isolated from bone marrow aspirate samples before treatment. PI-PLC beta1 gene expression was evaluated on peripheral blood samples from patients at baseline, and monthly until the 8th cycle of 5-AZA administration. **Results.** Between September 2008 and February 2010, 32 MDS patients with IPSS risk Low- or Int-1 were enrolled into the study. Most patients had a normal karyotype (63%) by metaphase cytogenetics, were BRC transfusion-dependent (81%), receiving a median of 4 units/mo, and were previously unresponsive to treatment including ESAs (69%). Twenty-six patients (81%) completed the treatment plan (8 cycles). The Overall Response Rate after the 8th cycle was 58% (15/26 pts) whereas 42% of patients maintained a stable disease; no patient progressed towards a high-risk MDS or AML. Five (19%) patients reached a complete remission whereas 10 (38%) achieved an hematological improvement. Transfusion independent was achieved in 8/26 patients (31%). The median duration of the response was 10 months; five patients maintain their response, that is CR in 2 cases (+24 and +30 months) and HI-E in 3 cases (+14, +25,+26 months) without any treatment or supportive therapy. All but one patients (14/15) who achieved an hematologic response during treatment with 5-AZA showed a statistically significant increase in PI-PLCbeta1 mRNA expression. In all patients the increase of PI-PLCbeta1 levels anticipated the clinical response obtained at the 8th cycle. SNP array karyotyping identified genomic abnormalities in all analyzed patients (100%) compared with only 9/26 (35%) with metaphase cytogenetics; although, SNP array-based assay has been demonstrated to enable the identification of genetic abnormalities in all analyzed patients, the type and the number of copy number abnormalities were not correlated with clinical response to 5-Aza. By contrast, LOH alterations showed to have a trend with stable disease. **Conclusion**s. The current results of our study showed that 5-Aza low-dose schedule may be a safety and effective treatment for low risk MDS pts and may induce durable responsesThe correlation between the hematologic response and the PI-PLCβ1 expression indicate that PI-PLCβ1 may be a realiable marker of response to AZA.

0322

RARE MUTATIONS OF SPLICING MACHINERY IN MDS WITH COMPLEX KAROTYPES

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Background. Myelodysplastic syndromes (MDS) are a heterogeneous group of chronic myeloid neoplasms showing a predisposition to acute myeloid leukemia (AML). Although a number of gene mutations have been reported in MDS, they are common in many myeloid neoplasms and therefore, the molecular pathogenesis that is unique to MDS has not been fully elucidated. In this point of views, the recent discovery of novel pathway mutations of the RNA splicing machinery in myelodyplasia provided a new insight into the pathogenesis of MDS. These splicing pathway mutations are frequent (45-85%) in, and largely specific to MDS and related myeloid neoplasms. However, currently, the relationship between these splicing pathway mutations and other common genetic alterations as well as their clinical impacts in MDS has not been elucidated. **Aims.** To explore the genetic interaction of splicing pathway mutations with other genetic lesions and its impact on clinical outcomes, we analyzed genome-wide copy number lesions and common gene mutations that may coexist with splicing pathway mutations in a set of 313 cases with myelodysplasia, using SNP array karyotyping, including 190 cases with target sequencing of analyzing next-generation sequencer about common gene targets in MDS. The effects of the splicing pathway mutations and other genetic lesions on survivals were also evaluated. **Results.** Splicing pathway mutations were identified in 182 (58%) among 8 components of the splicing machinery, which occurred in a mutually exclusive manner. SNP array karyotyping revealed 151 cases (48%) showing copy number alterations, in which 7q- and/or 5q- were the most frequent abnormalities. Interestingly, the splicing pathway mutations were found at a significantly lower frequency among patients with 7q- and/or 5q- ($p<0.0001$), where multivariate analysis revealed that 7q- and/or 5q- were independently and significantly associated with the lower frequency of spliceosome mutation ($p = 0.001$ for 7q- and $p = 0.029$ for 5q-). Although 7q- and/or 5q- with complex karyotypes were associated with a significantly poor prognosis ($p = 0.025$, log-rank test) as a whole, they did not seem to be a risk of poor prognosis among those patients carrying a splicing pathway mutation.In total, 216 mutations, 135cases were identified among 190 cases, including 49 *TET2* (26%), 34 *RUNX1* (18%), 28 *ASXL1* (15%), 29 *RAS* (15%), 24 *TP53* (13%), 19 *IDH1/2* (10%), 11 *CBL* (6%), 12 *EZH2* (6%) and 10 *ETV6* (5%) mutations. No specific association between splicing pathway mutations and other coexisting mutations, except for the higher number of coexisting mutations in *SRSF2*-mutated cases. **Summary and Conclusions.** Splicing pathway mutations are rare in MDS cases with complex karyotypes and predict better clinical outcomes in this subgroup.

0323

EFFECTS OF EXPERIMENTALLY INDUCED MITOCHONDRIAL FERRITIN OVEREXPRESSION IN NORMAL HUMAN ERYTHROID PROGENITORS AND IN THE K562 ERYTHROLEUKEMIC CELL LINE

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Background. In refractory anemia with ring sideroblasts (RARS), the iron deposited in the perinuclear mitochondria of ring sideroblasts is present in the form of mitochondrial ferritin (FtMt), which is expressed in the early stages of erythroid differentiation. However, it is unknown whether FtMt overexpression

is the cause or the result of mitochondrial iron deposition, and the mechanism of FtMt expression regulation remains unclear. **Aims.** Our aim was to investigate the possible influence of experimentally induced FtMt overexpression on erythroid differentiation and its capacity to induce a sideroblastic phenotype using a model system based on normal human hematopoietic progenitor cells. **Methods.** Lentivirus FtMt-transduced CD34+ bone marrow cells from 7 healthy donors were cultured for 21 days in a liquid medium with a cytokine cocktail according to a well established procedure that allowed the expansion of high numbers of erythroid progenitors and the *in vitro* production of erythrocytes. At various days samples of cultured cells were removed for biological studies including flow cytometry analysis to evaluate erythroid differentiation and STAT5 activation, ferritin expression, clonogenic assays, proliferative activity, apoptosis, heme content measurement and reactive oxygen species detection. In addition, the effect of the FtMt-transduction on iron metabolism and JAK2/STAT5 pathway activation was analyzed in K562 erythroleukemic cells. **Results.** Variable amount of FtMt (30-100 ng/mg of total proteins) was observed in FtMt-transduced cells, whereas FtMt protein was not detectable in wild type or GFP-transduced control cells. FtMt overexpressing normal erythroid progenitors were characterized by reduced cytosolic H ferritin levels and increased CD71 expression, indicative for cytoplasmic iron depletion, as well as by diminished heme content, and higher apoptotic rate, in comparison with the FtMt negative controls (P=0.0001). FtMt transduction did not inhibit proliferative activity nor did it abrogate cell ability to terminally differentiate, but the growth of clonogenic BFU-E was tendentially lower in FtMt-transduced samples; the appearance of Perls positive ring granules was noticed in rare late cells, after prolonged iron exposure. Significantly lower levels of STAT5 phosphorylation following Epo stimulation were found in FtMt positive erythroid progenitors compared to FtMt negative cells (P=0.03). On the contrary, no significant changes in comparison with the controls were observed after transduction with a mutant FtMt lacking ferroxidase activity. In the K562 cell line, FtMt overexpression reduced reactive oxygen species, STAT5 phosphorylation, and the anti-apoptotic Bcl-XL transcript, and determined cytosolic iron starvation, whereas the transferrin receptor 1 transcript increased due to the activation of the IRE/IRPs machinery. **Conclusions.** Experimental FtMt overexpression may modify mitochondrial iron availability, impair heme synthesis and trigger the pathogenic mechanism that leads to sideroblast formation and to ineffective erythropoiesis, at least in part, through the inhibition of the JAK/STAT pathway, which is central to erythroid differentiation. The antioxidant properties of FtMt might play a role in this inhibition. Thus, our data appear relevant to the pathophysiology of RARS. They have also considerable clinical implications, as they might provide the rationale for therapeutic strategies based on FtMt gene silencing of RARS progenitors to revert the pathological phenotype.

0324

THE ROLE OF AUTOPHAGIC CELL DEATH IN RIBOSOMOPATHIES

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Background. A significant percentage of myelodysplastic syndrome (MDS) patients carry a deletion in the 5q chromosome. Recently, 5q-MDS was classified as a "ribosomopathy", given the critical role of ribosomal protein gene S14 loss in this deletion. Ribosomopathies are characterized by hypoplastic anemia, bone marrow failure, and a predisposition to cancer (especially acute myeloid leukemia). In addition to 5q-MDS, ribosomopathies include Diamond-Blackfan anemia, Shwachman-Diamond syndrome, and some forms of dyskeratosis congenita. All these disease are linked to mutations in genes that function at various stages of ribosome biogenesis. While the loss of hematopoietic stem cells in these patients has conventionally been attributed to activation of the p53 tumor suppressor, several recent reports have alluded to the existence of a p53-independent mechanism of cell death. **Aims.** The aim of this work was to test the hypothesis that autophagy, the cellular process of self-digestion, is a p53-independent mechanism of death in cells with ribosome biogenesis impairments. **Methods and Results.** We used zebrafish models of ribosomal protein gene loss to determine that the widespread cell death observed is not associated with p53 stabilization or caspase activation, even upon DNA damage. Electron microscopy of these mutant embryos identified the presence of autophagosomes, and western blotting established the conversion of LC3-I to LC3-II (a hallmark of autophagy activation). These techniques in addition to confocal microscopy were then used to verify the activation of autophagy in human cells derived from ribosomopathy patients. **Conclusions.** Our results suggest that the p53-independent mechanism of death in cells with ribosome biogenesis impairments is linked to the activation of autophagy. We go on to discuss our prediction that acquired impairments in the function of autophagy may be linked to the malignant transformation of these patients to AML. This work suggests that autophagy plays an unappreciated role in the pathogenesis of ribosomopathies and may also shed light on why patients with these diseases are predisposed to malignancies.

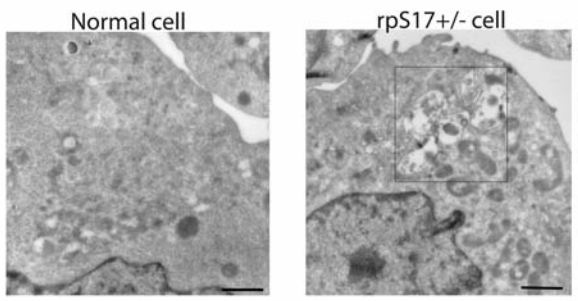

Figure 1. Autolysosomes (boxed area) are visible in ribosomopathy patient cells carrying an rpS17+/- mutation compared to normal controls.

0325

MASSIVE PARALLEL SEQUENCING OF T-CELL RECEPTOR BETA CHAINS REVEALS A MAINLY PUBLIC REPERTOIRE IN CHILDREN WITH VERY SEVERE APLASTIC ANEMIA

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Background. Very severe aplastic anemia (vSAA) is caused by the destruction of CD34+ bone marrow stem cells by autoreactive T-cells. The antigenic target on stem cells is still unknown and may vary between individuals. Only few putative pathologic T-cell clones have been identified yet. **Aims.** The general objective of this study was to comprehensively assess the overall diversity of the TCR repertoire of patients newly diagnosed with vSAA by a deep sequencing approach. Expanded TCR β sequences should be identified that may indicate candidate autoreactive T-cell clones associated with the pathogenesis of the disease. These findings should be correlated with patient-specific screens for autoreactive antibodies and gene expression analyses of CD34+ stem cells previously performed. **Methods.** High coverage, massive parallel Roche/454 sequencing of T-cell receptor β-chain amplicons was employed to analyze the T-cell receptor repertoire of bone marrow-derived CD8+ T-cells from children newly diagnosed with vSAA (n=7) and healthy controls (n=5). β-chain amplicons were characterized by automated analysis regarding gene segment usage, length and encoded amino acid sequence as well as sequence diversity. This information was integrated into a novel complexity score to assess overall T-cell diversity. **Results.** The TCR repertoire of vSAA patients was severely restricted and detected mainly as a low background of not expanded clonotypes. TRBV and TRBJ gene segment usage was different from normal individuals. A low number of specific TCR clonotypes frequently found also in healthy controls (so called "public"sequences) was dominantly expanded. 17% of all unique CDR3 amino acid sequences in vSAA patients were public sequences, in comparison, only 1.9% in normal controls. High coverage sequencing also identified several TCR β-chain sequences present exclusively in the cohort of vSAA patients that may indicate candidate clonotypes involved in the pathogenesis of the disease. In 4 of 6 patients integrin-reactive antibodies were detected and in 5 of 5 cases gene expression profiling revealed a significant downmodulation of integrin GPVI and the integrin complexes GPIa/IIa, GPIIb/IIIa, GPIB/GPIX/GPV on surviving CD34+ stem cells. **Summary and Conclusions.** T-cell receptor repertoire analysis based on next-generation-sequencing is a useful tool to evaluate and to monitor the restriction of patients' T-cell repertoire. In vSAA patients antigen recognizing sequences could be identified that may be involved in the autoimmune pathogenesis of the disease. The finding of an otherwise mainly public T-cell receptor repertoire in these patients may indicate a state of chronic immune stimulation that could finally lead to exhaustion and depletion of antigen-specific T-cells. It remains to be tested whether the dominant expansion of specific pub-

lic sequences, however, is associated with the pathogenesis of the disease. It may be speculated that common antigens may be overrepresented or pathologically presented in vSAA patients. Analyses of greater cohorts will be necessary to clarify whether these disease-associated clonotypes are rather patient-specific or structurally related and directed against common autoantigens such as integrin receptors targeted by auto-antibodies in these patients.

0326

KARYOTYPIC COMPLEXITY AND LOW PLATELET BASELINE COUNT PREDICT LENALIDOMIDE TREATMENT FAILURE IN MYELODYSPLASTIC SYNDROMES WITH DEL(5Q)

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Background. Deletion 5q is observed in 20% of karyotypically abnormal myelodysplastic syndromes (MDS) cases, and it is associated with good prognosis. Lenalidome is a drug effective in this subset of patients but 25% of the patients did not respond. However, not many genetic predictors of response to lenalidomide have been studied in MDS with deletion 5q.**Aim**In MDS 5q deleted patients, to identify differences between responders and non responders to treatment with lenalidomide.**Patients and methods**Fifty two MDS patients with 5q deletion treated with lenalidomide were selected. All were studied by conventional cytogenetics (CC) and SNP arrays with Genome-Wide Human SNP Array 6.0 (n=47) or GeneChip Human Mapping 250K Nsp Array (n=5). Most of them were studied by direct genomic sequencing for: *CBL*, *TET2*, *ASXL1*, *IDH1*, *IDH2* and *TP53* genes. Arrays results were verified by FISH in cases with available fixed Carnoy cells. Also, we assessed the copy number status of *TP53* by FISH.For treatment response (hematological) we considered two groups: responders (complete or partial remission, n=37) and non responders (stable disease, failure or progression, n=11). **Results.** By CC, 37 showed isolated 5q deletion, seven a deletion 5q + 1 aberration, and four a complex karyotype (≥3 alterations). Two cases were normal and two non informative with a deletion 5q detected by FISH.The deletion 5q was identified by SNP-A in all patients. It detected additional lesions in 27.03% of patients with isolated deletion 5q by CC. Two cryptic recurrent lesions were detected by SNP-A: deletion on 12p13.2-p13.1 and on 15q26.1. SNP-A identifies most of the 5q deletions starting at q14. All but one were interstitials; the CDR extended from q22.3 to q31.1 (120,438,462-134,989,521; 14.5 Mb) with a median deleted size of 69.7 Mb. FISH technique with commercial or BACs probes confirmed results (mainly losses) detected by SNP-A.Number of lesions detected by CC (0-1 *vs* ≥2) shows a significant correlation with treatment response (P=0.005), as well as, the 280x10^3/L platelet count cut-off point (P=0.006). Both variables were validated in a multivariate analysis (P=0.018 and P=0.036, respectively). However, additional aberrations detected by SNP-A seems not to be related with treatment response (P=0.288).Direct genomic sequencing identified one mutation in *CBL*, one in *IDH2*, eight in *TET2* and five in *TP53*. Mutations on *TP53* shows a trend to predict no hematological response (P=0.061). However, it is not related to cytogenetics response (P=0.150) but none of the mutated cases achieved complete cytogenetics response. Two of the mutated cases had *TP53* gene deleted in a low proportion (only detectable by FISH). None of them responded and one evolved to acute myeloid leukemia. **Summary.** Low karyotypic complexity (by CC) and a high baseline platelet count (>280x10^3/L) are associated with the achievement of hematological response. However, *TP53* mutations show a trend for lenalidomide treatment failure. **Acknowledgements.** Grants: FI 07/00107, PI 11/02010, RD07/0020/2004, R06/0020/0031, SGR 541, BES2008-008053, "PROMETEO"2011/025, Beca de Investigación de la Fundación Española de Hematología y Hemoterapia, and COST BM0801. CelgeneSpain.

0327

IMPAIRED CLEARANCE OF APOPTOTIC CELLS LEADS TO HMGB1 PROTEIN RELEASE IN THE BONE MARROW OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND INDUCES TLR4-MEDIATED PROINFLAMMATORY CYTOKINE PRODUCTION

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Background. Excessive pro-inflammatory cytokine production in the bone marrow (BM) has been recognized as a prominent pathogenetic mechanism for the disturbed hematopoiesis in patients with myelodysplastic syndromes (MDS). However, the upstream pathways, the exact cellular source and the triggering events related to cytokine excess in MDS BM remain unknown. **Aims.** To investigate the possible involvement of toll-like receptors (TLRs) and their endogenous ligands in the induction/maintenance of the inflammatory process in MDS BM. **Methods.** TLR expression was evaluated in the BM of MDS patients (n=27) and healthy controls (n=25) using flow-cytometry. Quantitative PCR analysis of 84 genes related to TLR-mediated signal transduction was performed using a commercially available PCR array in immunomagnetically sorted CD14$^+$ BM cells of patients and controls. Results were confirmed by testing separately three significantly over-expressed genes. The pro-inflammatory cytokine production by patient monocytes treated with autologous plasma in the presence/absence of a TLR4 inhibitor were measured with chemiluminescence and the levels of the high mobility group box 1 (HMGB1) protein, a TLR4 endogenous ligand, were assayed by ELISA in long-term BM culture (LTBMC) supernatants. To examine whether the excess of HMGB1 in patients' BM is due to macrophage dysfunction we used a fluorescent microscopy-based assay and we estimated the macrophage capacity to phagocytose apoptotic cells. We also assessed the HMGB1 levels in co-cultures of patient macrophages with different numbers of apoptotic cells. **Results.** A statistically significant increase in the proportion of TLR4$^+$ cells within the CD14$^+$ BM cell fraction was observed in patients (6.10%±5.27%) compared to controls (2.01%±1.38%) (P <0.0001) along with an up-regulation of TLR4 expression as was indicated by the TLR4 MRFI in MDS patients (P=0.0002). Fifty-three out of 84 TLR-related genes displayed at least a four-fold increase mRNA expression in MDS patients compared to controls. The relative mRNA expression of MyD88, TRIF/TICAM1 and TRAM/TICAM2 was significantly increased in MDS patients (2.39±1.26, 2.23±2.28 and 0.08±0.03, respectively) compared to controls (0.76±0.43, 0.89±0.60 and 0.01±0.009, respectively) (P=0.0001, P=0.0159 and P<0.0001, respectively). Incubation of patient monocytes with autologous BM plasma resulted in a TLR4-dependent production of proinflammatory cytokines as was indicated by the significant decrease of IL-1β, IL-6 and TNFα levels in the presence of the TLR4 inhibitor compared to cultures treated with the BM plasma alone (P=0.0313, P=0.0313 and P=0.0313, respectively). HMGB1 levels were significantly increased in LTBMC of patients compared to controls suggesting that HMGB1 might constitute an endogenous TLR4-activating ligand in MDS BM. Patient-derived macrophages displayed impaired capacity to engulf apoptotic cells (12.00%±2.00%) in comparison to healthy individuals (36.70%±4.81%; P=0.0079). HMGB1 release by MDS BM macrophages loaded with increasing numbers of apoptotic cells for different time periods was dependent on the apoptotic cell load (P<0.001) and incubation time (P=0.0417). **Summary and Conclusions.** A primary apoptotic cell clearance defect of BM macrophages might contribute to the inflammatory process in MDS BM through aberrant release of TLR4-inducing molecules such as HMGB1, from the apoptotic/necrotic cells present in patients' marrow microenvironment.

0328

EZH2 MUTATION INTERACTS CLOSELY WITH ASXL1 AND AML1/RUNX1 MUTATIONS IN PATIENTS WITH *de novo* MYELODYSPLASTIC SYNDROME

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Background and Aims. Mutations of the Enhancer of Zeste Homologue 2 (*EZH2*) gene, which encodes the histone methyltransferase, were recently

found in patients with myelodysplastic syndrome (MDS). The clinical and prognostic relevance of these mutations in MDS are unclear. **Methods.** A total of 272 *de novo* MDS patients diagnosed according to French-American-British (FAB) criteria at the National Taiwan University Hospital who had cryopreserved bone marrow cells for study were recruited into mutational analyses. Mutations in *EZH2* gene at exon 2-20 were analyzed by polymerase chain reaction and direct sequencing. The results were correlated with clinical features, cytogenetics, other gene mutations and treatment outcomes. **Results.** Among the 272 patients, 94 patients (34.6%) had refractory anemia (RA), 16 (5.9%) had RA with ring sideroblasts (RARS), 94 (34.6%) had RA with excess blasts (RAEB), 32 (11.8%) had RAEB in transformation (RAEBT), and 36 (13.1 %) had chronic myelomonocytic leukemia (CMMoL) according to the FAB classification. The disease of 204 patients fulfilled the criteria of MDS according to 2008 WHO classifications. *EZH2* mutations were detected in 17 (6.3%) of the 272 patients diagnosed according to the FAB classification and in 7(3.4%) of the 204 according to 2008 WHO classification.*EZH2*-mutated patients had higher WBC counts at diagnosis than *EZH2*-wild patients (median 8840/μL *vs.* 3915/μL, P=0.002). According to FAB classification, patients with CMMoL had the highest incidence (22.2%) of *EZH2* mutations, followed by RAEBT (6.3%), RA (4.3%), RAEB (3.2%), while none of the RARS patients had this mutation (P=0.001). There was no difference in the distribution of 2008 WHO classification, karyotype, and international prognostic scoring system (IPSS) between patients with and without *EZH2* mutations.To investigate the interaction of gene mutations in the pathogenesis of MDS, a mutational screening of 10 other genes was also performed. Among the 17 patients with *EZH2* mutations, 15 (88.2%) showed additional molecular abnormalities at diagnosis including eight with concurrent both *ASXL1* and *AML1/RUNX1* mutations, five *ASXL1* mutation, one *ASXL1* and *FLT3*/ITD and one both *ASXL1* and *NRAS* mutations. All but one patient with *EZH2* mutation showed concomitant mutation of *ASXL1,* a gene involved in the regulation of histone methylation, compared with 22.3% in those without *EZH2* mutation (P<0.001). The patients with *EZH2* mutation also had a significantly higher incidence of *AML1*/*RUNX1* mutation than those with *EZH2*-wild type (50.0% *vs.* 11.5%, P<0.001).With a median follow-up of 61months (range, 0.1-250.7 months), there was no significant difference in overall survival between patients with and without *EZH2* mutation by either FAB or 2008 WHO classifications (18.7 months *vs*. 30.5 months, P=0.493 and 33.8 months *vs*. 44.6 months, P=0.348, respectively). Subgroup analyses in patients with different-cytogenetic risk and in patients with different IPSS scores could not demonstrate prognostic impact of *EZH2* mutation, either. **Summary and Conclusions.** The highly close association of *EZH2* mutation with *ASXL1* mutation suggests that the two mutations may interact with each other in the pathogenesis of MDS. Further studies are necessary to disclose the mechanism of their concerted action in the development of MDS.

0329

SERUM IL-10 LEVELS AT DIAGNOSIS CORRELATE WITH HEMOGLOBIN CONCENTRATION, TRANSFUSION NEED AND OVERALL SURVIVAL IN PATIENTS WITH LOWER-RISK MYELODYSPLASTIC SYNDROMES

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Background. Some 15% of MDS patients present with a hypocellular bone marrow. This fact has driven research towards eventual underlying immunological mechanisms, in analogy to severe aplastic anemia. Immune-induced cell damage and increased apoptosis might not be mutually exclusive, but even causally linked, and immune response may explain the clinical effectiveness of immunomodulators in some MDS patients. **Aims.** The aim of this study was to analyze serum levels at diagnosis of 6 cytokines and 3 chemokines in lower-risk MDS patients, as well as to evaluate their correlation with hemoglobin concentration at diagnosis, erythrocyte transfusion need during their first 16 weeks of follow-up, and overall survival (OS). **Methods.** Eighty-three lower-risk (IPSS<1.5) MDS patients (51 M and 32 F), prospectively recruited at 8 spanish GESMD sites and classified according to WHO-2008 (RCUD 5, RARS 6, RCMD 50, RAEB-1 12, 5q- syndrome 9, unclassifiable 1) were studied. The study was approved by the IRB at each study site and all patients gave written informed consent. Cytological diagnosis and cytogenetic evaluation followed standard operating procedures of the GESMD. Age, gender, hemoglobin, serum albumin, LDH, ferritin, beta2m, EPO, IL-2p70, IL-1beta, IL-10, IL-8, TNF-alpha, MIG, MCP1 and IP-10 levels (ELISA) were evaluated at diagnosis. Lee's Comorbidity Index (LCI, JAMA 2006) and the number of erythrocyte concentrates transfused in the first 16 weeks from diagnosis were also collected. Median follow-up was 17.9 months (range 4.4-35.2, IQR 11.4-21.4). During this time, 6 patients (7.2%) progressed to acute leukemia, 12 (14.5%) died and 4 (4.8%) were lost to follow-up. Kaplan-Meier estimates were calculated by using median as cutoff, and Logrank test for contrast. When median values were statistically significant, Logrank test-for-trend, using p25, p50 and p75 values, was also calculated. **Results.** According to their very network relationship, most of cytokines/chemokines levels were correlated among them. Serum levels of IL-6, IL-10, MIG and IP-10 showed a statistically significant correlation with age, LCI, albumin and beta2m, but strikingly only IL-10 correlated as well with Hb at diagnosis (Rho=-0.378; p<0.001) and transfusion need during the first 16 wks (+0.379; p<0.001). IL-1beta, TNF-alfa and IP-10 serum levels showed a negative correlation with LDH serum levels, but none of the cytokines/chemokines analyzed correlated with serum ferritin or EPO levels at diagnosis. Kaplan-Meier analysis showed that both EPO and IL-10 serum levels (see figure), but not IL-6, TNF-alfa or other cytokines/chemokines predicted OS in this lower-risk population. The mechanisms underlying the observed association are unclear, but might be mediated by endogenous GM-CSF production suppression and subsequent BFU-E growth inhibition, that seem to explain rHuEPO resistance in some CRF Patients (Cooper AC, J Am Soc Nephrol 2003; Oehler L, Blood 1997).The prognostic value of IL-10 serum levels seems independent of IPSS, but warrants further investigation in a larger series of patients. **Conclusions.** IL-10 serum levels correlate with hemoglobin concentration, transfusion need and OS in lower-risk MDS patients. This effect seems independent of EPO serum levels.*Spanish MDS Group (GESMD). This study was supported by a grant from Celgene, S.L., Madrid (project INBIOMED HEMA-001/2006).*

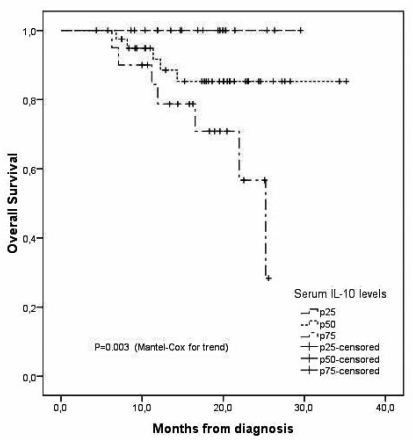

Figure 1.

0330

MYELODYSPLASIA TO ACUTE LEUKAEMIA: THE COMBINATION OF MICROARRAY GENE EXPRESSION DATA AND SHRNA KNOCKDOWN SCREENS TO UNDERSTAND THE PROGRESSION OF MDS TO AML.

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Myelodysplastic syndromes (MDS) are bone marrow disorders resulting in ineffective haematopoiesis. However, a significant subset of patients will transform into Acute Myeloid Leukaemia (AML), which typically has a very low short-term survival rate. This study has identified and modulated genes whose expression changes prior to AML transformation to understand the molecular mechanisms that drive it.Microarray data, a subset of the international MILE study, from patients with a diagnosis of MDS was used to develop a gene expression-based prognostic classifier which identified 3 MDS groups with low (>36 months), intermediate (18-36 mo.) and high (<18 mo.) risk of AML transformation. This study has examined microarray gene expression across five groups (the three risk groups with the addition of normal bone marrow and normal/complex karyotype AML) using ANOVA. Genes were selected whose expression varied significantly across the groups (FDR-adjusted p-value <0.05) and also consistently increased or decreased with progression. A second microarray dataset from Pellagatti et al. 2006 that analysed the $CD34^+$ component from MDS and healthy bone marrow was used as a validation study.The overlap between these expression studies identified 343 genes, several of

which had previously established roles in *de novo* AML, such as HOX genes and WT1. However, several genes were identified whose potential role in leukaemia or MDS to AML progression had yet to be characterised. Pathway analysis of these novel genes suggested both central and peripheral involvement in non-canonical ubiquitin signalling. The gene list was further refined by examining only those genes that showed a high expression in AML and MDS cell lines. An shRNA knockdown screen (with at least three shRNA per gene) of these genes in those same cell lines has reinforced the significance of a deregulated ubiquitin pathway in MDS to AML progression.This study highlights the ubiquitin pathway in the transformation of MDS to AML providing an innovative therapeutic target for MDS patients at risk of transformation.

0331

SINGLE NUCLEOTIDE POLYMORPHISM ARRAY (SNP-A) KARYOTYPING IN LOWER RISK MYELODYSPLASTIC SYNDROMES (MDS), A STUDY BY THE GROUPE FRANCOPHONE DES MYÉLODYSPLASIES (GFM)

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Background. Single nucleotide polymorphism arrays (SNP-As) allow the identification of chromosomal defects undetected by metaphase cytogenetics (MC), such as acquired copy-neutral loss of heterozygosity (CN-LOH) and offers higher resolution of unbalanced chromosomal defects. Myelodysplastic syndromes (MDS) are characterized by recurrent chromosomal abnormalities and molecular lesions that predict outcomes. **Aims.** A genome-wide scanning to identify known and new cryptic genomic abnormalities in a cohort of lower-risk MDS patients selected for their resistance to erythroid stimulating agents and prospectively treated in a phase 2 study, comparing treatment with azacitidine alone or in combination with epoetin-beta. **Patients and methods.** We performed Affymetrix SNP6.0 array analysis on 79 low-risk and intermediate-1 MDS patients, all enrolled in the GFM azaepo2008-1 study (NCT01015352). Median age was 70 years (range 42-85). Diagnosis according to WHO were RARS (n=36), RCMD-RS (n=14), RCMD (n=7), RAEB (n=10), RA (n=5), CMML (n=6) and 1 unclassified. MC results were available for 78 patients (one failed) and 55 (71%) had a normal cytogenetics (NC). **Results.** We identified 37 copy number abnormalities: 15 gains (median 146Mb; range: 1Mb-243Mb), and 22 losses (median 23Mb; range: 1.2Mb-78Mb). The most recurrent gain observed was trisomy of chromosome 8 (n=10). Losses preferentially concerned chromosomes 5 (n=4), 20 (n=4) and Y (n=3) and 4 (n=2). Thirteen CN-LOH were detected, most frequently on chromosomes 4 and 14, with a median size of 73Mb (range:2Mb-117Mb). Overall, 22 patients had one SNP-A lesion, 8 had 2 and 4 had 3 lesions. Regions of losses and/or CN-LOHs contained likely candidate genes (i.e. *DNMT3A, TET2, CBL, ASXL1* and *RUNX1*). Combining MC and SNP-A karyotyping lead to higher diagnostic yield of chromosomal defects as compared to MC alone (44% vs 29%;p=0.06). Indeed, additional chromosomal lesions were identified by SNP-A in 20% of patients with NC by MC and in 27% of patients with abnormal MC. MC did not detect sub-clonal trisomies 2 and 12, and partial duplications of chromosomes 2 [dup(2)(q12.3q14.3)] and 19 [dup(19)(p13.3p13.3)]. One monosomy 19 and two large deletions of chromosome 13 [del(13)(q14.13q22.1)] and 20 [del(20)(q11.22q13.2)] were similarly not detected by MC. In the remaining six NC by MC, microdeletions of a size ranging from 1.2Mb to 2.2Mb were identified by SNP-A. In addition, analysis of the patient with unsuccessful MC by SNP-A enabled the detection of a microdeletion encompassing *TET2* gene. SNP-A analysis also reveals 5 patients with unfavorable cytogenetics (4 complex karyotypes and one del(7q)) compared to none by MC. If we assume that genomic aberrations detected by SNP-A carry a similar prognostic value to those detected by MC, six patients by SNP-A analysis should then be switched to a higher IPSS risk category. Deep sequencing of previously known and new genes mutations is in process in this cohort of lower risk MDS patients to allow better risk-stratification with combined techniques. **Conclusions.** Our study demonstrated that SNP-A reveals unrecognized chromosomal abnormalities that may explain the clinical heterogeneity of lower-risk MDS patients. Use of SNP-A in clinical practice could improve prognostic classification and ensure a more appropriate management of individual patients.

0332

GLOBAL DNA METHYLATION PREDICTS THE RESPONSIVENESS TO AZACITIDINE IN HIGH-RISK MYELODYSPLASTIC SYNDROMES

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Background. Simultaneous promoters hypermethylation of CpG nucleosides in DNA is believed to be a key contributor to the molecular pathophysiology of Myelodysplastic Syndromes (MDS) by inactivating genes involved in the control of normal cell growth, differentiation, apoptosis, adhesion and cell motility. Aberrant DNA hypermethylation is associated with a poor prognosis in MDS that can be accounted for by more rapid progression to Acute Myeloid Leukemia (AML).Epigenetic therapy using DNA hypomethylating agents, such as azacitidine, is clinically effective for the treatment of MDS. **Aims.** We investigated the association between global DNA methylation in MDS patients with clinical outcome. Results were compared to an age-matched control group of healthy subjects and to a group of *de novo* and secondary AML patients. Then we focused on the role of global DNA methylation to predict the clinical response to azacitidine. **Methods** Bone marrow biopsy and mononuclear cells from 128 patients affected by MDS, comprising all subgroups, and 14 patients with high risk MDS treated with azacitidine were examinated. Immunochemistry was performed on paraffin-embedded sections using anti-5-methylcytosine/5mc antibody. Double immunostainings were performed for nuclear 5-methylcytosine/5mc and one of four cytoplasmic/cell membrane markers: CD34 for precursors, MPO for myeloid cells, Glycophorin-C for erythroid cells, Factor VIII for megakaryocytes. LINE-1 sequences were measured by COBRA methodology and by Pyrosequencing technology. **Results.** Our results showed that in MDS the global DNA methylation was intermediate between normal controls and AML and significantly correlated with age, blast count and karyotype.Moreover we identified at multivariate analysis only global methylation and age as significant independent prognostic factors affecting overall survival (OS).When we evaluated the global DNA hypermethylation in patients treated with azacitidine, we observed that it decreased in 10 responder patients and anticipated the haematological response, whereas it didn't in 4 patients with stable disease. **Conclusions.** Our data confirmed that DNA hypermethylation in MDS correlates with the clinical outcome. Also, we demonstrated, not only that global DNA methylation could be used to monitor the effect of azacitidine, but also may anticipate the haematological response. A longer follow-up is needed to make any correlation with prognosis and overall survival.

0333

SINGLE NUCLEOTIDE POLYMORPHISM ARRAY (SNP-A) KARYOTYPING: A DIAGNOSTIC AND PROGNOSTIC TOOL IN PRIMARY MYELODYSPLASTIC SYNDROMES WITH UNSUCCESSFUL CONVENTIONAL CYTOGENETIC TESTING

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Background. Myelodysplastic syndromes (MDS) are a group of acquired clonal hematopoietic disorders, characterized by cytopenias, dysplasia in one or more of the myeloid cell lines and an increased risk of development of acute myeloid leukemia. Cytogenetic abnormalities identified by metaphase cytogenetics (MC) are observed in approximately 50% of MDS cases, and have an important diagnostic and prognostic role. In some MDS patients MC may fail to provide results because of the growth of none or few metaphases, with the

consequently loss of its important prognostic information. Fluorescence *in situ*hybridization (FISH) may complement the MC study, however, its utility is limited to the detection of particular lesions. SNP-A-based karyotyping, that only requires DNA extraction for its performance, could resolve MDS cases with non-informative MC. **Aims.** To apply SNP-A karyotyping in MDS patients with an unsuccessful routine cytogenetic to search for genomic aberrations and to study the potential usefulness of this technique to improve the diagnosis and prognostication of these patients. **Methods.** We studied 40 patients with primary MDS whose bone marrow (BM) karyotype showed no mitoses (n=38) or less than 10 analyzed metaphases (n=2). Diagnosis of MDS was made according to the 2008 WHO classification (Table 1). DNA was extracted from peripheral blood (PB) (n=14) or BM (n=26) at time of diagnosis. Samples were processed with the Affymetrix®Genome-Wide Human SNP Array 6.0. **Results.** SNP-A results are summarized in Table 1. SNP-A analysis enabled the detection of aberrations in 19 (47.5%) patients. Seven out of 19 aberrations were detected in PB samples and 12 in BM ones. SNP-A abnormalities were most frequently found in chromosomes 3, 5, 7 and 11. In five cases we observed a complex karyotype (≥3 numerical aberrations) by SNP-A. We observed four cases involving deletion of 5q (all confirmed by FISH) and three cases with 7q affected (a deletion and two CN-LOH). Median follow-up was 8.4 months (range 1-83.1). Median overall survival for the entire cohort was 67.8 months (95% CI 11.2-124.4). We analysed the survival of patients with CNAs detected by SNP-A and it was worse compared to those in whom no CNA were detected (median 7.7 months [95% CI: 6.4-9.0] *vs.* 67.8 months [95% CI: 0.5-135.1]; *P*=0.003). We tentatively assessed the IPSS risk group of the 35 patients with clinical and morphological data available, using the CNAs information obtained by SNP-A to determine the cytogenetic risk category. We clustered the patients into low/intermediate-1 risk and intermediate-2/high risk and we observed differences in survival between the two groups (OR 5.87 [95%CI 1.64-21]; *P*=0.007). **Conclusions.** SNP-A could be used successfully in PB samples. The identification of CNAs by SNP-A would improve the diagnostic and prognostic evaluation of MDS patients with non informative MC. Recurrent areas of acquired CN-LOH are lesions widely presented in MDS, their definitive impact awaits a formal prognostic analysis in larger series.This work was supported by grants from the ISCIII, Ministerio de Sanidad y Consumo, Spain (FI07/00107, PI07/1009 and PI 11/02010); RTICC (FEDER RD07/0020/2004), SGR 541, Acción COST BM0801, INBIOMED HEMA-001/2006 and Celgene Spain.

Patient	Age	Sex	Diagnosis (WHO 2008)	Sample	Metaphase cytogenetics	SNP-A data		
						Loss	Gain	CN-LOH
1	70	M	RCUD	PB	No mitoses	No	No	3q25.1qter (MLF1, TERC, THPO, DBA5) 11ptel|15.5
2	78	M	RCMD	PB	No mitoses	No	No	4p15.32p13 6q23.2q26 (MYB) 7q11.2q34
3	70	M	RCMD	PB	No mitoses	No	No	No
4	83	M	MDS-U	PB	No mitoses	3ptel|24.3 (FANCD2) 5q12.3q33.3	No	No
5	78	F	RCMD	PB	46,XX[8]	No	No	No
6	75	M	RAEB-1	PB	No mitoses	No	No	No
7	73	M	RCMD	PB	No mitoses	No	No	No
8	89	M	RAEB-1	PB	No mitoses	5q13.1q35.3 7q22.1q36.3 13q13.1q13.3 13q14.13q21.2 (RB1) 13q31.3q32.2 16q11.1q21.2 (BCL6, CDH1) Chr.18	1p36.33p36.13 13q13.3q14.13 13q31.3q31.3	17ptenq12
9	78	M	RCMD	PB	No mitoses	No	No	No
10	92	F	RCUD	BM	No mitoses	5q22.1q33.3	No	No
11	76	M	RCMD	PB	No mitoses	No	No	2p26.3p26.1 2q31.1q35 (CASP10) 5p15.2p12 10p15.3p14 16p13.1p13.3
12	64	M	RCMD	PB	No mitoses	No	No	No
13	76	M	RARS	PB	No mitoses	No	No	No
14	77	M	RCMD	PB	No mitoses	No	No	3q25q26 (MLF1)
15	77	F	RCMD	BM	No mitoses	No	No	No
16	90	F	RAEB-2	BM	No mitoses	No	No	No
17	78	F	RCMD	PB	No mitoses	No	Chr. 8	No
18	67	M	RCMD	BM	No mitoses	No	No	No
19	88	M	RCMD	BM	No mitoses	No	No	No
20	72	F	RAEB-2	PB	No mitoses	No	No	No
21	65	F	RCMD	BM	No mitoses	No	No	11q14.1q23.1
22	86	M	RCMD	BM	No mitoses	No	No	No
23	58	M	Unknown	BM	No mitoses	No	No	No
24	88	M	RCMD	BM	No mitoses	No	No	No
25	88	M	RCMD	BM	No mitoses	No	Chr. 8	No
26	75	F	Unknown	BM	No mitoses	No	No	No
27	39	F	Unknown	BM	No mitoses	No	No	No
28	78	M	Unknown	BM	No mitoses	No	No	1p31.3p21.3 2p16.3p13.3 4qtel|16.1 9ptel|24.2
29	74	F	RARS	BM	No mitoses	No	No	No
30	70	M	Unknown	BM	No mitoses	No	No	No
31	89	F	RCMD	BM	No mitoses	No	No	7q11.2q22.2 12ptel|13.31 14q22.2q24.3
32	67	F	RAEB-2	BM	No mitoses	3q25.33q25.33 5q14.3q23.1 5q23.1q33.3 12q13.31q21.3	9p24.1p24.1	11q
33	81	M	RAEB-1	BM	No mitoses	16p22.1q22.2 20q11.23q13.2	No	No
34	67	F	RAEB-1	BM	No mitoses	4q21.2q28 5q11.2qter 7p12.1p11.2 7q11.22q11.22 7q11.21qter 12q21.2q21.2 13q13.3q21.2 13q21.2q21.31 13q34q34 17q13.3q11.2	3q25.1q28	No
35	61	M	RAEB-1	BM	No mitoses	7ptel|12.1 11q14.1qter Chr. X	No	No
36	88	F	RAEB-2	BM	No mitoses	No	No	No
37	68	M	RAEB-1	BM	No mitoses	21q22.12q22.12	No	No
38	76	M	RCMD	BM	No mitoses	7q	1q	No
39	70	F	RCMD	BM	No mitoses	5q22.1q31.3	No	No
40	90	F	RCMD	BM	46,XX[2]	No	No	No

Abbreviations: M: male; F: female; RCUD: refractory cytopenia with unilineage dysplasia; RCMD: refractory cytopenia with multilineage dysplasia; MDS-U: myelodysplastic syndrome unclassified; RAEB: refractory anaemia with excess of blasts; RARS: refractory anaemia with ringed sideroblasts; PB: peripheral blood; BM: bone marrow; CN-LOH: copy neutral loss of heterozygosity.

Figure 1.

0334

THE PROLIFERATION INHIBITOR CDKN1C (P57/KIP2) IS EXPRESSED IN A LEFT SHIFTED HEMATOPOIETIC STEM CELL COMPARTMENT AND PREDICTS POOR OUTCOME OF MDS

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Purpose. Progressive cytopenias are the cause of death for the majority of patients with Myelodysplastic syndromes (MDS). In order to investigate if proliferative activity of CD34+ cells in MDS bone marrow correlates with prognosis, we measured expression of the proliferation inhibitor CDKN1C (P57KIP2). **Patients and Methods.** Two independent patients cohorts were recruited for investigating mRNA expression profiles (n=183) and p57 protein expression in immunohistochemistry of bone marrow biopsies (n=93) of patients with MDS. Overall survival was correlated with CDKN1C/P57KIP2 expression. **Results.** In purified CD34+ cells, mRNA expression of p57kip2 correlated with higher risk and poorer overall survival of patients with MDS (p=0.0006, n=183). Furthermore, increased CDKN1C expression was associated with loss of CD38 as well as loss of cyclins and cyclin-dependent kinases underlining the role of p57kip2 as a proliferation inhibitor within a left shifted stem cell compartment. Similarly, protein expression of P57KIP2 in immunohistochemistry independently predicts poor prognosis of patients with MDS (p=0.0003, n=93). This marker also has prognostic impact in RAEB-2 patients and after AML-like chemotherapy. In contrast, P57KIP2 does not predict outcome after allogeneic stem cell transplantation. Multivariate cox regression analysis with the confounding co-variates: age, cytopenias, cytogenetic risk profile, blast count and primary versus secondary MDS confirmed the impact of P57KIP2 expression on OS. Most importantly the interaction term P57KIP2 expression and blast count did not remain significant in the final model, providing evidence that P57KIP2 expression is an independent risk factor. P57KIP2 protein expression could also be demonstrated in sorted CD34+ cells of MDS patients and was significantly higher expressed than in CD34- cells. **Conclusions.** Low CD38 and high p57kip2 expression in CD34+ cells suggest a left shifted hematopoietic stem cell (HSC) compartment with a reduced fraction of proliferative and increased numbers of dormant HSC. Exhaustion of proliferative and accumulation of dormant HSC may therefore form the basis of the progressive cytopenias of late, non-leukemic MDS.

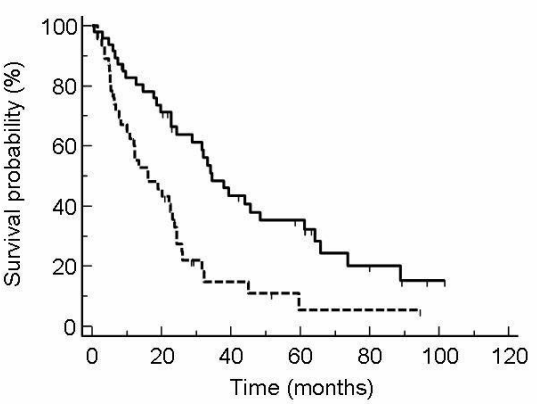

Figure 1 Impact of p57 expression in trephine biopsies on overall survival in patients with MDS.

0335

DISTINCT PHENOTYPIC CHARACTERISTICS OF MDS WITH DEL(5Q) ABNORMALITIES MEASURED BY 8-COLOR FLOW CYTOMETRY

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Background. Patients (pts) with myelodysplastic syndrome (MDS) harbouring a deletion of the long arm of chromosome 5 (del(5q)) often display characteristic morphological features in the bone marrow (BM). Besides, 4-colour based flow cytometry (FCM) has become a valuable tool in the general diagnostics of pts with MDS, although it is still unknown, whether aberrations detected by FCM differ between certain cytogenetic subtypes. **Aims.** Using an 8-color FCM diagnostic panel we aimed to investigate potential differences in antigen patterns between MDS pts with and without del(5q) abnormalities. **Methods.** BM samples of 123 MDS pts with either del(5q) (IPSS low/int-1=32, int-2/high=25) or non-del(5q) MDS (IPSS low/int-1=50, int-2/high=16) were investigated. FCM was performed on a FACS Canto and standardized including the analysis of all samples after overnight storage and erythrocytes lysis prior to antibody staining. Thresholds were set according to median ± 2SD and/or ½ log differences compared to healthy BM samples. FCM score (FCSS) by Wells et al. 2003 and modified by van de Loosdrecht et al. 2008 into different groups (0-1 low, 2-3 moderate, >3 high) was applied. **Results.** When comparing low/int-1 MDS pts significantly more del(5q) pts had a moderate FCSS (67% vs. 31%; p=0.013) and less pts expressed aberrant lymphoid antigens (48% vs. 75%, p=0.048). Within granulopoiesis del(5q) pts showed less abnormalities (p=0.028), a higher lymphoid-to-myeloid ratio (p=0.021) reflecting a higher lymphocyte (19% vs. 10%, p=0.002) as well as a lower granulocyte (62% vs. 72%, p=0.009) count, and a higher degree of hypogranularity (p=0.023). Of note, within the monocytes significantly less del(5q) pts presented with aberrant lymphoid CD56 antigen expression (19% vs. 53%, p=0.014). Whereas aberrant CD56 expression on blasts was not different within IPSS low/int-1 pts, it was exclusively present in int-2/high del(5q) MDS vs. non-del(5q) MDS pts (63% vs. 0%, p<0.001). Interestingly, the amount of B-lymphoid progenitor cells (lyPC) was higher indel(5q) compared to nondel(5q) MDS (4.0% vs. 2.0%, p=0.030) and myeloid progenitors (myPC) showed a lower MFI for CD13 (p=0.033). Considering low/int-1 MDS with "isolated del(5q)"only, the above mentioned differences were even more pronounced: e.g. lymphoid-to-myeloid/monocytic ratio (p=0.003; p=0.018), higher grade of hypogranularity of granulocytes (p=0.006), and higher percentage of lyPC (p=0.002).Furthermore, during disease monitoring phenotypic characteristics such as percentage of CD34+ myPC, abnormal lymphoid-to-myeloid ratio, CD10- granulocytes or CD71 expression in erythrocytes disappeared in patients with a cytomorphological response. By contrast, characteristics like decreased CD19 or aberrant CD56 expression in CD34+ cells or percentage of CD45++ lymphocytes were rather unaffected by lenalidomide treatment. **Conclusions.** Eight-colour based FCM is a valuable tool in the diagnostics of MDS. It might also be able to distinguish del(5q) MDS from other MDS subtypes. The value of flow cytometric disease monitoring remains to be further analysed.

0336

OXIDATIVE STRESS CAN INFLUENCE GENE METHYLATION STATUS IN MDS PATIENTS

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Myelodysplastic syndromes (MDS) are a heterogeneous stem cell bone marrow disorders characterized by the underproduction of one or more blood cells types, due to hematopoiesis dysfunction, and higher risk of leukemic transformation. Our previous results and indirect evidences from other studies suggest a role for oxidative stress (OS) in MDS etiology and pathogenesis. OS, resulting from an imbalance between Reactive Oxygen Species (ROS) production and antioxidant defenses, contributes to cell damage, apoptosis and ineffective hematopoiesis. On the other hand, our previous studies also show that CDKN2A and CDKN2B, two closely linked tumor suppressor genes involved in cell cycle regulation, which encode, respectively, for p16 and p15 cyclin-dependent kinase inhibitors, are inactivated by methylation. Although the cause of altered patterns of DNA methylation in cancer remains unknown, oxidative stress might influence DNA methylation profile.In this context, we investigate the relation between oxidative stress and p15 and p16 methylation status as risk factors and prognostic marker in MDS patients.For this purpose we have examined the expression levels of ROS, peroxide and superoxide anion, and the antioxidant defense GSH, in CD34 bone marrow cells collected at diagnosis from 24 patients with *de novo* MDS. The expression of these oxidative stress parameters was evaluated by flow cytometry using the fluorescent probes, H2DCF-DA, DHE and mercury orange, respectively. Genomic DNA was isolated from bone marrow aspirates by standard protocols and modified by sodium bisulphite. The MS-PCR for p15 and p16 promoter methylation was performed using two sets of primers, one for methylated DNA and other for unmethylated DNA. The patient group median age was 77 years (33-84), gender M/F=13/11, WHO subtypes: RCMD (n=8), RA (n=5), RAEB-1 (n=2), RAEB-2 (n=6), 5q- syndrome (n=1), LMMC (n=2) and IPSS: low (n=6), intermediate-1 (n=13) and intermediate-2 (n=5).Our previews results show that CD34 MDS cells had higher expression of peroxide, although a significant lower expression of superoxide anion and GSH levels, compared to controls. However, ROS and GSH expression were subtype-dependent (EHA09). On the other hand, 50% of MDS patients have at least one methylated gene and 21% have both methylated genes. MDS patients who have at least one methylated gene show a significant increase in peroxide expression levels (565±699 MFI) when compared with patients with p15 and p16 unmethylated genes (230±352 MFI). This fact is substantiated in patients with both methylated genes (878±743 MFI). Beside that, superoxide and GSH levels were lower in patients with at least one (O_2^{-}, 180±109 MFI; GSH, 165±136 MFI) or both methylated gene (O_2^{-}, 183±130 MFI; GSH, 156±143 MFI), compared patients with unmethylated genes (O_2^{-}, 256±136 MFI; GSH, 331±569 MFI). Moreover, patients with high peroxide levels have an increase frequency of gene methylation (43%) and a lower survival, compared to patients with normal/low levels (12%).This study suggests a correlation between oxidative stress, mainly peroxide levels, and gene methylation profile in MDS patients, since MDS patients with higher peroxide levels have higher frequency of gene methylation and lower survival.*This work was supported by CIMAGO (Project 22/09).*

0337

PTEN/PI3K/AKT PATHWAY DYSREGULATION IN MYELODYSPLASTIC GRANULOCYTES

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Background. Patients with Myelodysplastic Syndromes are at increased risk of infections not only because of the reduced number of granulocytes, but also for their functional abnormalities. Myelodysplastic neutrophils were shown to present impaired fungicidal and bactericidal activities and reduced chemotaxis. PTEN/PI3K/AKT pathway plays a crucial role in mediating cellular response to chemotactic stimuli. **Aims.** To study gene expression of PTEN/PI3K/AKT signaling pathways in myelodysplastic granulocytes compared to the normal counterpart. **Methods.** We evaluated PTEN mRNA expression in peripheral blood granulocytes isolated by Ficoll gradient centrifugation and osmotic erythrolysis from 30 patients with MDS and 12 normal controls by Sybr Green-based real-time RT-PCR, using Abl as reference gene. Data were expressed as $2^{-\Delta Ct}$. DNA methylation of two regions of the PTEN promoter (from -1223 bp to -1123 bp and from -300 bp to -128 bp) was studied by Methylation-specific PCR on bisulfite-treated DNA from 19 MDS and 6 controls. The gene expression profile of 84 genes belonging to the PTEN/PI3K/AKT signaling pathway was studied on granulocytes from 10 MDS patients and 10 matched normal control using the Human PI3K-AKT Signaling Pathway RT² Profiler® PCR Arrays (SABiosciences, Qiagen). Patients' cohort used for PCR arrays included the following MDS subtypes: 2 RCMD, 2 RAEB1, 4 RAEB2, 1 CMML and 1 t-MDS. Selected housekeeping genes were GAPDH, RPL13A and HPRT1. Data analysis was performed using the RT² Profiler® PCR Array Analysis Template v3.3. **Results.** PTEN mRNA was significantly down-regulated in myelodysplastic versus normal granulocytes (median 157,59 and 401,22, respectively, *p*=0.029). No significant differences in PTEN mRNA levels were observed in different MDS subtypes. PTEN promoter was not methylated in MDS and control granulocytes. Ten genes belonging to the PTEN/PI3K/AKT signaling pathway were found to be significantly down-regulated in MDS versus controls, including NFKBIA, FOS, AKT1, MAPK3, RBL2, RPS6KA1, GRB2, PIK3CG, RAF1 and ADAR (fold change less than -2 and p<0.05). Among studied genes, the most significantly suppressed was NFKBIA (fold change: -5,63, *p*=0.004) which encodes for the NFKB inhibitor-α, whose reduction might contribute to deregulated signaling across the PI3KT-AKT pathway in myelodysplastic granulocytes.**Summary/ Conclusions.** Deregulation of PTEN/PI3K/AKT signaling pathway can contribute to granulocytic dysfunction in MDS.

0338

INCIDENCE OF 17P DELETION AND TP53 MUTATION IN MYELODYSPLASTIC SYNDROME AND ACUTE LEUKEMIA WITH 5Q DELETION.

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Background. Del(5q) is the most frequent cytogenetic abnormality in myelodysplastic syndrome (MDS) and is associated with good prognosis when isolated or associated with only one other abnormality, on the other hand del(5q) in complex karyotype is associated with an increase risk of leukemic transformation. Recently, *TP53* mutations were found in 18 % of MDS with isolateddel(5q) and no excess of marrow blasts, especially in patients failing to respond or progressing after response to Lenalidomide. **Aims.** To assess the incidence of *TP53* mutations detected by direct sequencing and of del(17p) detected by Fluorescence *in situ* hybridization (FISH), their correlation and prognostic value in a cohort of Myelodysplastic Syndromes, and Acute Myeloid Leukemia (AML) withdel(5q). **Methods.** Bone marrow of 43 patients with MDS or AML anddel(5q), including 20 with isolateddel(5q) (or with one additional abnormality), and 23 with complex karyotype were selected. Three patients were also studied during follow up. **Results.** In patients with isolated del(5q) or one additional abnormality, no 17p deletion was found by FISH and 3 of the 18 cases analyzed (17 %) had *TP53* mutation. In patients with complex karyotype, 17 p abnormalities including del(17p), der(17) or monosomy 17 were observed by conventional cytogenetic (CC) in 15 of 23 cases, *TP53* deletion was confirmed by FISH in 10 of them. *TP53* mutation was found in 6 of the 13 patients tested (46%), only 3 of whom had del(17p). In the whole patient series, *TP53* mutations were associated with shorter survival (p=0.07)**. Conclusions.** We confirm the existence of *TP53* mutations in 17% of MDS with isolated del(5q). In patients with del(5q) and complex karyotype, FISH and sequencing are complementary techniques to analyze *TP53* abnormalities Our findings further suggest that, in MDS with del(5q), those abnormalities may occur in a defined order, with initial mutation of one *TP53* allele, followed by deletion of the second allele through del(17p), the latter event generally concomitant with appearance of a complex karyotype. These results encourage us to pursue *TP53* studies in larger cohort to determine patients with better chance to respond to Lenalidomide treatment.

0339

LENALIDOMIDE INFLUENCES THE FUNCTIONAL CAPACITY OF MESENCHYMAL STROMAL CELLS IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background. Lenalidomide (LEN) has been introduced as an effective drug capable of generating transfusion independency in a high percentage of low to intermediate-1 risk myelodysplastic syndrome (MDS) patients. While the direct mechanisms of action of the drug on the malignant cells have been intensively studied, its effects on the stromal compartment of the bone marrow (BM) have not been investigated so far systematically. **Aims.** Therefore, we investigated the in-vitro effects of LEN on human mesenchymal stromal cells (MSC) as a relevant cellular component of the BM microenvironment and if drug treatment influences functional capacities of these cells. **Methods.** Primary MSC from MDS patients (n=17) with different subtypes, including MDS-5q, and age matched healthy donors were incubated with LEN at concentrations of 0.1 and 1 µM for every experiment. Vitality and proliferation were quantified by Trypan Blue and MTT assay, respectively. Apoptosis (Annexin V staining) and cell surface marker expression were investigated by FACS. Cells were submitted to osteogenic and adipogenic induction to study differentiation capacity. A Boyden chamber assay was used to test the migration capacity of primary CD34+ hematopoietic cells towards conditioned medium of LEN treated or untreated MSC, and the concentration of SDF-1 in these supernatants were determined by ELISA. The MSC support of hematopoietic CD34+ cells was tested a coculture assay quantifying cobble-stone forming area cells after 4 weeks (CAFC). The ability of treated and untreated MSC to sensitize KG-1A cells to TNF-alpha was investigated by Annexin V staining after 24 h of coculture. **Results.** We could demonstrate that normal as well as MDS-MSC treated with LEN at clinically relevant concentrations retain their ability for proliferation, adipogenic and osteogenic differentiation and expression of distinct cell surface markers. The SDF-1 secretion by MSC was significantly reduced in healthy donors and MDS-5q groups, and this correlated with decreased induction of migration of CD34+ cells. The supportive capacity for hematopoiesis was also affected by LEN as demonstrated by a significantly lower number of CAFC after 4 weeks. MDS-MSC treated with LEN sensitized leukemic blasts (KG-1A cell line) to TNF-a induced apoptosis in direct coculture. **Conclusions.** Our in-vitro study suggests that LEN treatment at therapeutically relevant concentrations does not affect the proliferation and differentiation capacity of MSC from healthy donors and patients with MDS. Important functional activities of MSC were influenced by LEN: support of hematopoietic cells and SDF-1 secretion were reduced, and induction of TNF-alpha sensitivity of KG-1A cells cocultured with MDS-MSC, a defective feature in MDS, was rescued. These findings point to an indirect mechanism of action of LEN in MDS possibly via modulation of the microenvironment.

0340

ACUTE MYELOID LEUKEMIA (AML) ARISING FROM THE GPI-NEGATIVE HEMATOPOIETIC CELL POPULATION IN A PATIENT WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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Background. PNH is a rare acquired disorder of the hematopoietic stem cell (HSC), characterized by a somatic mutation in the X-linked *PIGA* gene. This results in deficiency on the cell membrane of all proteins anchored by the glycosylphosphatidylinositol (GPI) molecule. In all PNH patients a clone arising from a GPI-negative (*PIGA*-mutant) HSC has undergone expansion. Although one might regard this very fact as a pre-leukemic condition, the estimated frequency in PNH of hematological malignancies, mostly AML, is between 0.6% to 2.9% of patients. In PNH most of hematopoiesis is supported by the GPI-negative clone, but residual GPI-positive cells exist in most cases: therefore it is not trivial to ask whether leukemia arises from within the GPI-negative clone or not: until now this has been determined only in very few cases. **Aims.** To identify the origin of leukemia arisen in a patient with classic hemolytic PNH. **Methods.** Phenotype and genotype of leukemic cells have been investigated by flow-cytometry and DNA/RNA sequencing. **Results.** *Case report*. A 71 years old former stonemason developed anemia, and was found to have PNH, with a large PNH clone (GPI-negative granulocytes: 98%). Neutrophil and platelet counts were normal. A bone marrow aspirate revealed erythroid hyperplasia and a normal karyotype. High reticulocyte counts, very high LDH levels and occasional hemoglobinuria were consistent with classic hemolytic PNH. The patient required approximately 3 units/month of packed-RBC. Just over 2 years after PNH diagnosis an increase in transfusion requirement (up to 6 units/month of packed-RBC) was noted, and there was evidence of iron overload. Six months later the patient developed acute leukemia (WBC 11,000/µL with 84% blasts in peripheral blood) with an immature myeloid phenotype: CD34+CD33+CD38+CD13+CD117+CD71+HLA-DR+CD7+. The patient was offered palliative cytoreductive treatment and unfortunately died within weeks. *Molecular studies*. Sequence analysis of the *PIGA* gene on DNA from PNH granulocytes before leukemia onset revealed a mutation in exon 2 (715G>T). This mutation predicts an amino acid replacement G239W; in addition, since this mutation destroys the canonical "donor site"at the distal end of exon 2, we anticipated it would affect splicing. Indeed, PCR amplification of mRNA revealed an aberrant *PIGA* transcript resulting from the use of a cryptic "donor site"(548 of intron 3). The translation of this aberrant transcript results in a non-functional truncated protein (118 amino acids) that is responsible for the GPI-negative phenotype. *Studies on the leukemic blasts*. All blasts were negative for the GPI-linked molecule CD59, indicating that leukemia arose from within the PNH clone. On DNA obtained from leukemic blasts we found exactly the same exon 2 mutation that was present in granulocytes before the leukemia onset. **Comments.** There are only a handful of cases in the literature in which it has been shown that AML in PNH arose from within the PNH clone. To the best of our knowledge, this is the first case in which this is formally proven by detecting the same mutation in leukemic blasts and in non-leukemic granulocytes from the same patient before he developed AML.

0341

BALANCE OF NK, NKT CELLS AND MAIN CYTOKINES - POSITIVE AND NEGATIVE REGULATORS OF HAEMOPOIESIS - IN PATIENTS WITH APLASTIC ANAEMIA AT DIFFERENT STAGES: RELAPSE AND REMISSION

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Background. In the basis of pathogenesis of aplastic anaemia (AA) lays immune-mediated disturbance of haemopoiesis, related to dysbalance of cytokines, produced by immune cells, including NK and NKT cells. A possible role of these cells in development of aplasia of hemopoiesis in AA patients now is being broadly studied. Nevertheless, their level and, especially, dynamics during the disease course are not well established yet. Considering that normally haemopoiesis is provided by balanced complex of positive and negative cytokine signals, the AIM of our study was to determine ratio of main opposite cytokines produced by mononuclear cells of AA patients, and their relations with levels of NK and NKT cells at different stages of the disease: relapse and remission. **Methods.** We have examined 23 patients with AA in dynamics. Percentage of NK and NKT cells was evaluated using flow cytometry (Beckman Coulter, FC500). Production of cytokines was evaluated in supernatants of peripheral blood mononuclear cells after 24-hour incubation at 37°C using ELISA. We have studied levels of the following cytokines: IL-1β and IL-6 (positive factors), TNFα (negative regulator of haemopoiesis), IL-4 (TNFα antagonist) and IL-2 (T-cell growth factor). **Results.** The level of natural killer cells in peripheral blood was 9,78±1,9%. that was significantly lower than norm (13,7±1,0%), while NKT cell level was 11,1±2,1% being 2-fold higher than norm (5,5±0,7%). Relapse of the disease was characterized by significant decrease of NK cells to 4,98±1,2% and increase of NKT cells up to 10,1±2,3%. In remission NK cells increased up to 12,40±0,9%, while level of NKT cells decreased to 9,15±1,3%, nevertheless being statistically significantly higher than normal. We have revaeled that normally the intensity of synthesis of positive haemopoietic factor IL-6 prevails over synthesis of other cytokines. At the same time, in AA priority of synthesis of hemopoietic factors shifts towards inhibitory cytokine - TNFα, which production exceeds normal level (44.2 pg/mL) more than 10-fold. Relapse of the disease is characterized by progression of all disturbances in cytokine balance, which were revealed in AA. Thus, significant increase of IL-1β and IL-2 production is accompanied by extremely increased, dominating over all other cytokines, intensity of synthesis of negative hemopoetic regulator - TNFα (18-fold higher than normal) and decreased production of its antagonist - IL-4. Complete clinical-hematological remission is characterized by a tendency to normalization of cytokine ratio. Thus, during remission there occurs a shift in priority of cytokine production from negative regulator - TNFα towards IL-6. The intensity of synthesis of this positive factor begins to dominate over other cytokines, similar to that revealed in norm. Nevertheless, even during complete clinical-hematological remission a production of TNFα by mononuclear cells remains increased. **Conclusions.** The fact that increased amount of NKT cells in relapse of AA was accompanied by marked increase of TNFα and decrease of IL-4, and that decrease of NKT cells in remission was accompanied by decreased TNFα and increased IL-4 can be the evidence of a role that NKT cells play in pathogenesis of AA.

Myelodysplastic syndromes - Clinical 1

0342

EPIGENETIC REGULATION AND DEREGULATION OF VAULTRNA1-3 IN MDS

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Introduction. The role of non-protein encoding RNAs (ncRNAs) in normal cellular function has been widely recognized in the past decade, and perturbations in their expression can have great consequences in the initiation and progression of cancer. Also, epigenetic marks are essential for normal gene regulation and have a profound role in the pathogenesis of cancer. We have recently shown that a specific ncRNA, of the class of vaultRNAs (vtRNA2-1), is a prognostic factor in AML (Treppendahl et al., Blood, 2012). This project aims to investigate the epigenetic regulation and deregulation of the three other vtRNA members in myelodysplastic syndrome (MDS). **Materials, Methods and Results.** Treatment of the myeloid cell line, HL60, with demethylating drugs (azacytidine and decetabine) showed that vtRNAs are regulated by DNA methylation, and that vtRNA1-3 could be cancer-specifically silenced by DNA methylation. vtRNA1-3 is located in a region commonly deleted in MDS (5q33.3), indicating that it may function as a tumor suppressor. Initial analysis of the vtRNA1-3 methylation status in MDS patients (n=54, mixed IPSS groups) did not yield a significant difference in survival between patients with hypermethylated or unmethylated vtRNA1-3 promoters (P=0.15). However, analysis of the patients within the low and intmerdiate-1 risk groups (n=26), did show that patients with vtRNA1-3 hypermethylation did have a decreased survival compared to patients with unmethylated alleles (P=0.02), indicating that vtRNA1-3 may be involved in the pathogenesis of low/intermediate-1 risk MDS.Secondly, this project investigates the overall epigenetic structure of the vtRNA promoter regions. vtRNAs are RNA polymerase III transcripts, and have a unique combination of promoter elements not seen elsewhere in the genome. Our preliminary results indicate that vtRNAs have a unique and unexpected positioning of nucleosomes at their promoters and that histone modifications may also regulate their expression. **Conclusions.** These initial studies show that epigenetics can regulate the expression of vtRNAs, and that aberrant DNA methylation of the vtRNA1-3 promoter may be involved in the pathogenesis, and prognosis, of low and intermediate-1 risk MDS.

0343

FISH ANALYSES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) AND HEALTHY CONTROLS SUGGEST THAT THE LOSS OF A Y CHROMOSOME RESULTS FROM A COMBINATION OF AGE-AND DISEASE-RELATED FACTORS

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Background and Aims. An isolated loss of the Y chromosome is associated with a very good prognosis in myelodysplastic syndromes (MDS). Remarkably, the prognosis in these patients is better than that of a normal karyotype, even if the mean age in the affected group is higher as compared to the typical MDS cohort (Ganster et al. ASH 2010). Despite this, it is still a matter of debate whether the loss of the Y chromosome is an age-related phenomenon or a clonal abnormality in MDS. Thus, the main goal of the study presented here was to examine this question in a multicentric study. **Methods.** We hypothesized that a disease related Y-loss would be present in CD34+ clonal cells, but not in CD3+ T-cells not belonging the MDS clone. Therefore, we determined the frequency of cells with Y-loss in CD34+ and CD3+ cells of MDS patients and of control persons not suffering from hematopoietic diseases. CD34+ and CD3+ cells were enriched by immunomagnetic or fluorescence activated cell sorting (FACS). The frequency of cells with Y-loss was determined by Fluorescence In Situ Hybridization (FISH) analysis. We analyzed CD3+ and CD34+ peripheral blood cells of 23 MDS patients (median age 78 years) and CD3+ peripheral

blood cells of 25 younger (median age 27 years) and of 25 elderly (median age 74 years) control persons. Additionally, CD34+ bone marrow or peripheral blood cells of 10 elderly control persons (median age 73 years) were analyzed so far. **Results.** In elderly MDS patients, the number of cells with Y-loss was significantly increased in CD34+ compared to CD3+ cells. The median clone size was 51% (range 8%-97%) in CD34+ cells and 5% (range 1%-14%) in CD3+ T-cells (p<0.01). Regarding the fact that MDS is extremely rare in young men below 35 years, we were not able to analyze the differences in CD3+ versus CD34+ cells in this group. Analyzing healthy controls, we found that a Y-loss in CD3+ cells of young men below the age of 35 is very infrequent. The median frequency was 0.5% (range 0%-2%), as compared to 2% (range 0%-14%) in CD3+ cells of elderly men (p<0.01). In the latter group, a Y-loss was observed in 8% (median) of CD34+ cells (range 2%-16%). To date, the analysis of CD34+ cells from the peripheral blood of young healthy men is pending. **Conclusions.** The absence of Y-loss in CD3+ cells of young healthy men compared to up to 14% Y-loss in CD3+ cells of elderly men implies an age related Y-loss in T-cells. Y-loss in up to 16% of CD34+ cells of healthy controls suggests an age-related Y-loss in CD34+ cells too. To improve diagnostic accuracy, we now aim to establish an age-matched laboratory threshold for the Y-loss in CD34+ cells. We conclude that the Y-loss in MDS patients is not just age-related or clonal but often a combination of both.

0344

CHRONICALLY TRANSFUSED MYELODYSPLASTIC SYNDROMES (MDS) PATIENTS HAVE SEVERE IRON BURDEN: DEFERASIROX TREATMENT REDUCES IRON OVERLOAD AND IMPROVES LIVER FUNCTION

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Background. Diagnosis and monitoring of iron overload, and the effect of iron chelation therapy in MDS patients, is often based on serum ferritin (SF) assessment, which is easily measured and accessible for most patients. Limited data on liver iron concentration (LIC) assessment exist in MDS, primarily due to biopsy-related risk of bleeding and infections in these patients. LIC is a more clinically robust and direct measure of body iron burden, and with increasing availability of non-invasive magnetic resonance imaging (MRI) techniques to measure LIC, it is becoming more practical to use in the management of transfused MDS patients. **Aims.** This pooled analysis assesses the degree of iron burden in a large cohort of MDS patients, focusing on LIC assessments in patients enrolled and completing 1-year deferasirox treatment. Relationships between LIC, SF and alanine aminotransferase (ALT) (a marker of liver function) are also examined. **Methods.** Data were pooled from iron-overloaded patients with MDS having Low/Int-1 risk or a life expectancy >1 year and who were enrolled in four open-label single-arm deferasirox studies. LIC was assessed in three of four studies using R2-MRI. In one study, LIC was assessed by ultrasound-guided percutaneous liver biopsy or by magnetic liver susceptometry using a superconducting quantum interference device (SQUID). LIC values obtained by SQUID were multiplied two-fold to correct for the underestimation of LIC by SQUID compared to biopsy (Porter *et al. EJH* 2008). Correlations were evaluated using Pearson's correlation coefficient.

Table 1. LIC, SF, and ALT parameters after 1-year deferasirox treatment in patients with MDS.

	n	Baseline	EOS	Change from baseline
Mean ± SD LIC, mg Fe/g dw				
Overall	71	20.5 ± 14.6	13.9 ± 13.1	−6.6 ± 10.3
Baseline LIC <7 mg Fe/g dw	15	4.1 ± 1.6	5.1 ± 2.5	1.0 ± 2.8
Baseline LIC ≥7 mg Fe/g dw	56	24.9 ± 13.4	16.2 ± 13.7	−8.6 ± 10.7
Median serum ferritin (range), ng/mL				
Overall	71	2620 (538 to 12,639)	2035 (158 to 10,520)	−630 (−9188 to 4834)
Baseline LIC <7 mg Fe/g dw	15	1593 (954 to 4950)	1938 (733 to 7073)	278 (−2630 to 4834)
Baseline LIC ≥7 mg Fe/g dw	56	2745 (538 to 12,639)	2036 (158 to 10,520)	−851 (−9188 to 3162)
Mean ± SD ALT, IU/mL				
Overall	71	55.9 ± 37.8	38.9 ± 27.7	−17.0 ± 36.7
Baseline LIC <7 mg Fe/g dw	15	29.4 ± 22.9	38.1 ± 27.3	8.7 ± 26.3
Baseline LIC ≥7 mg Fe/g dw	56	63.0 ± 38.0	39.1 ± 28.1	−23.9 ± 36.2

Results. Overall, 124 patients had LIC assessment at baseline (57.3% males; mean age, 65.4 years). Majority of patients had severely elevated LIC (<7 mg Fe/g dw, 15.3%; ≥7-≤15 mg Fe/g dw, 28.2%; and >15 mg Fe/g dw, 56.5%). SF levels were also elevated (baseline SF: ≤2500 ng/mL, 48.4%; >2500-≤5000 ng/mL, 35.5%; and >5000 ng/mL, 16.1%).Of the 124 patients, 71 patients also had end-of-study (EOS) LIC measurements, allowing efficacy assessment after 1-year deferasirox treatment. Mean deferasirox dose was 19.6 ± 6.5 mg/kg/day. Mean baseline LIC was 20.5 ± 14.6 mg Fe/g dw. In patients with baseline LIC ≥7 mg Fe/g dw, LIC was reduced by -8.6 ± 10.7 mg Fe/g dw by EOS, and 58.9% of patients achieved an LIC decrease of ≥30%. SF and ALT levels also decreased (Table 1). In patients with LIC ≥7 mg Fe/g dw, 32.1% had normal ALT levels at baseline, improving to 71.4% by EOS.At baseline, LIC and SF had a moderately strong correlation (R=0.546; P<0.001). Baseline LIC and ALT correlated to a lesser extent (R=0.361; P=0.002). Change in LIC correlated with change in both SF (R=0.336; P=0.0042) and ALT (R=0.397; P=0.0006). **Summary and Conclusions.** LIC was severely elevated in a high proportion of this large cohort of transfusion-dependent MDS patients. LIC decreased and the proportion of patients with normal baseline ALT levels increased after 1-year deferasirox treatment, particularly in patients with baseline LIC ≥7 mg Fe/g dw. Reductions in SF and ALT correlated with those of LIC. These results indicate that reduction of moderate-to-severe iron overload may be associated with improved liver function in MDS patients.

0345

PROGNOSTIC ANALYSIS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA TRANSFORMED FROM MYELODYSPLASTIC SYNDROMES: WHO CAN BENEFIT FROM WHICH THERAPY?

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Background. Myelodysplastic syndromes (MDS) often transform into acute myeloid leukemia (AML). Such transformed patients (AML-MDS patients) have genetic backgrounds different from that of *de novo* AML patients. Risk-scoring systems, such as the International Prognostic Scoring System (IPSS) and the WHO classification-based prognostic scoring system (WPSS), are used to predict AML transformation in MDS. However, no established data exist to help predict the survival of AML-MDS patients. Similarly, there are few data on selecting available therapies, such as conventional chemotherapy (CC), allogeneic stem cell transplantation (allo-SCT), and best supportive care (BSC). **Aims.** To analyze the survival after the transformation in AML-MDS patients, in particular in association with treatment before and after transformation. **Methods.** We retrospectively analyzed data from 136 patients with AML-MDS at 4 different centers from Japan, Hungary and The Netherlands. Their initial diagnoses at the MDS stage included 17 refractory cytopenia, 5 refractory anemia with ringed sideroblasts, 42 refractory cytopenia with multilineage dysplasia, 63 refractory anemia with excess blasts , and one 5q- syndrome. Univariate survival analysis as a function of clinical variables was performed using the Kaplan-Meier method combined with the log-rank test. The variables included parameters at initial diagnosis and at AML transformation (e.g., age, sex, blood cell counts, blast percentages in the bone marrow, karyotype, IPSS, and WPSS) and treatment before and after transformation. Multivariate analysis for survival was performed using Cox proportional hazards regression analysis. **Results.** In univariate analysis, age, percentage of blasts in peripheral blood, and performance status (PS) at AML transformation as well as application of CC before AML were associated with survival time after AML transformation in all patients. In multivariate analysis, older age and higher percentages of blasts in peripheral blood at transformation were associated with significantly shorter survival. Age had the most significant effect on survival when the patients were divided into age ≥ 60 years (n = 106, older group) and < 60 years (n = 30, younger group). In the older group, 47 patients received CC, 46 received BSC, 6 received allo-SCT, and 7 received other therapies. The choice of therapy did not affect the survival time in this group. However, in the younger group, allo-SCT (n = 16) showed superior survival time compared with CC and BSC (P < 0.0001). **Conclusions.** Older age and high percentages of circulating blasts at AML transformation were poor prognostic factors. The IPSS and WPSS at the MDS stages and karyotypes at AML transformation did not predict the survival after the transformation. Furthermore, cytotoxic therapies, except for allo-SCT in the younger group, showed little survival benefit.

0346

RECOMBINANT HUMAN ERYTHROPOIETIN (EPO) IN VERY ELDERLY PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes (MDS) are the most common hematologic malignancy in elderly patients and their onset is usually around the age of 70. Recombinant human erythropoietin (EPO) has been widely used to treat anemia in lower risk MDS patients, but few data are known about EPO treatment in the very elderly patient group. **Aims of the study** The objective of this study was to investigate the role of EPO treatment in terms of response, overall survival and toxicity in a very elderly MDS patient group. **Methods.** Ninety-three MDS patients (M/F 57/36) who were treated with EPO when aged ≥ 80 years were selected among MDS cases enrolled in a retrospective multicenter study by the cooperative group GROM (Gruppo Romano Mielodisplasie) from 01/2002 to 12/2010. Diagnosis was made according to FAB and WHO Criteria; responses were evaluated according to IWG criteria (2006). The IPSS score was used for prognostic stratification. **Results.** Median interval from diagnosis to EPO start was 2.8 months (IR 0.7 - 10.2). At EPO start, median age was 82.8 years (IR 81.2 - 84.7) with a mean haemoglobin level of 8.8 g/dl (SD +/-0.98). According to FAB classification, there were 61 (65.5%) patients with RA, 6 (6.5%) with SA and 26 (28.0%) with RAEB. According to WHO classification, there were 15 (16.1%) patients with RA, 2 (2.1 %) with SA, 39 (41.9%) with RCMD, 4 (4.2%) with RCMD-S, 17(18.3%) with RAEB-1, 9 (9.6%) with RAEB-2 and 7 (7.8%) with isolated del5q. The IPSS score was calculated in 60 patients with an available karyotype: 28 (46.6%) patients were low-risk, 26 (43.3%) int-1 and 6 (10.1%) int-2. Creatinine level was normal in 73 (78.5%) cases and elevated in 20 (21.5%) cases; 40 patients (43.0%) had a previous transfusion requirement. Median serum EPO level was 37.1 mU/L (IR 22.1 - 75.0). The initial dose of EPO was 40.000 UI/week in 34 (36.5%) patients and 80000 UI/week in 59 (63.5%) patients. An erythroid response was observed in 62 (66.6%) patients, which was major in 54 (58.0%) and minor in 8 (8.6%). No thrombotic event was reported during the treatment. Predicting factors for erythroid response were hemoglobin level at baseline > 8 g/dl (p = 0.03), ferritin levels < 250 ng/ml (p=0.007) and no previous transfusion requirement (p < 0.001). On the other hand, serum EPO levels at baseline < 50 mU/l (p=0.199) and normal creatinine levels (p=0.138) were not predictive for response. Median overall survival from EPO start was 48 months (CI 95% 27,5 - 68,4) in responders versus 30.6 months (CI 95% 7,3 - 53,8) in resistant patients (p= 0.185). Overall, toxicity was observed only in 4 responders.**Conclusions** Our data outline that EPO treatment is safe and effective also in very elderly MDS patients. However, further prospective and larger studies are warranted to confirm these data and to evaluate if EPO treatment could be worthwhile in terms of quality of life and cost-efficacy in very old patients.

0347

CYTOGENETIC CHARACTERIZATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) FROM 'ANDROMEDA' NETWORKING PROJECT

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Background. Cytogenetic evaluation of bone marrow samples in myelodysplastic syndromes (MDS) is a very important step not only to confirm the diagnosis but it is invaluable in defining the classification, the prognosis, the expected survival, as well as the risk of progression to acute myeloid leukemia (AML). The validation of cytogenetic risk groups based on WHO categories may provide useful information to define model for risk stratification.*Objective:* We aimed to improve focus on the value of cytogenetic evaluation in classification, staging and in order to predict clinical outcomes in MDS. In this way we developed a pilot clinical project to offer a service of conventional cytogenetic analysis for any patients with a suspected MDS that were examined from 11 medical centres of the Puglian Haematology Network. This pilot project was called ANDROMEDA that is an Italian acronym of ANalysis of cytOgenetics alteRatiOn in the MyEloDysplAstic syndromes. Another aim of the Andromeda service will be create a collaborative network between the medical centres involved in order to integrate their experience and to select risk-adapted stratification in MDS. **Methods.** We analyzed the cytogenetic and clinical characteristics from consecutive *de novo* MDS patients (n=146) admitted in medical centers between January 2011 and December 2011. All bone marrow tissues were collected from patients with suspected MDS and they were sent by the treating physician to a skilled Central Cytogenetic Laboratory to undergo classical and FISH cytogenetic analysis. Peripheral blood, bone marrows smears and core biopsy slides from all cases were reported in a case report form (CRF) through a central database in anonymity . All patients gave their informed consent to participation. **Results.** Overall there were 75 (51.4%) men and 71 women (48.6%) included in the ANDROMEDA program and median age was 74 years. The overall incidence of clonal chromosome abnormalities was 38.2%. Identified single abnormalities included del(5q) isolated (9 cases, 17.0%), +8 (6 cases, 11.3%), -7 (2 cases, 3.8%), nevertheless majority of 5q-, 8+, -7 cases combined with additional cytogenetics abnormalities (32.0%, 24.5% and 13.2% respectively). Complex karyotypes were detected in 12 cases (22.6% of abnormal karyotype). Only 6 (4.1%) bone marrow samples were not unqualified for cytogenetic analysis. Patients were classified in WHO subtypes as 27.5% refractory anemia (RA), 9.2% refractory neutropenia (RN), 2.5% refractory anemia with ring sideroblasts (RARS), 30.8% refractory cytopenia with multilineage (RCMD), 10.8% refractory anemia with excess of blast-1 (RAEB-1), 11.6% refractory anemia with excess of blast-2 (RAEB-2), 4.2% MDS with deletion 5q (MDS 5q-) and 3.3% MDS unclassifiable (MDS-U). **Conclusions.** These preliminary data demonstrated the feasibility and effectiveness of a project like Andromeda. The data collected may be associated with environmental and genetic factors of its territory in such a manner as to constitute a valid basis for epidemiological studies on MDS.

0348

TELOMERASE GENE SCREENING AND TELOMERE OVERHANG DETECTION IN CHINESE PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background. Human telomeres are the long DNA TTAGGG repeats at the ends of chromosomes. Telomere dynamics and telomerase expression are fundamentally involved in cellular aging and cancer. The telomeric 3'-G rich overhang is a crucial telomeric structural component responsible for the t-loop formation. Loss of telomerase function also leads to short telometric overhangs, potentially resulting in chromosome instability. Shortened telomeric overhang was noticed in aplastic anemia patients with telomerase gene mutations and seem to correlate with poor response to immunosuppressive therapy. **Aims.** To

better understand the role of telomerase gene mutations and overhang length in the progression of myelodysplastic syndrome. **Methods.** We screened bone marrow samples from 62 Chinese patients with myelodysplastic syndrome (MDS) for variants in telomerase reverse transcriptase(TERT) and telomerase RNA component(TERC) gene. We also investigated the length of telomeric overhangs in those patients and 46 healthy individuals, using Southern blot analysis. Cytogenetic status, disease severity and short time survival rate in patients with MDS were evaluated. **Results.** Our cohort included 16 RA, 6 RAS, 2 RCMD, 15 RAEB I, 14 RAEB II, They were 36 males and 26 females, age was 56 (17-79), medium follow-up time was 12 months (4-36 months). All patients were evaluated with IPSS and WPSS scoring system. Neither TERT nor TERC mutations was identified in patients with MDS. Telomeric overhang length decreased as IPSS or WPSS value increased (Tables 1 and 2). Patients with higher risk IPSS or WPSS had shorter telomere overhang length compared to those with lower risk. Multivariate regression analysis showed telomeric overhang length, as well as IPSS and WPSS, is an independent prognostic factor for patients with MDS (IPSS P=0.001 overhang P=0.02; WPSS P=0.001 overhang P=0.012). **Conclusions.** Telometic overhang lengths change in accordance with IPSS and WPSS in patients with MDS. Short overhang length may be an independent factor for poor response and shorter survival in patients with MDS. These findings would have to be confirmed in large, prospective studies.

Table 1. Telomere overhang length in MDS patients with different IPSS.

	No. of cases	Overhang length Average ± SD	P value
Low risk*	24	200.9±70.9	NS between *and **
Intermediate I**	18	189.7±55.1	
Intermediate II***	8	138.2±32.9	NS between *** and ****
High risk****	12	133.1±30.1	
A= *+**	42	196.1±64.1	P=0.0001 when compare A and B
B= ***+****	20	135.1±30.5	

NS: No significant difference.

Table 2. Telomere overhang length in MDS patients with different WPSS.

	No. of cases	Overhang length Average ±SD	P value
1. Very low risk (score 0)	8	193.2±32.8	NS between 1 and 2 and 3
2. Low risk (score 1)	23	190.4±67.9	
3. Intermediate risk(score 2)	6	252.6±54.1	
4. High risk (score 3-4)	19	146.9±35.8	NS between 4 and 5
5. Very high risk (score 5)	6	112.1±46.8	
A=1+2+3	37	201.1±63.0	P<0.0001 when compare A and B
B=4+5	25	140.0±39.8	

NS: No significant difference

0349

EVALUATION OF AZACITIDINE IN TRANSFUSION-DEPENDENT, EPO-REFRACTORY PATIENTS WITH LOWER-RISK MDS

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Background. Transfusion-dependency (TD) in patients with low- and int-1-risk MDS is associated with increased morbidity and mortality (Malcovati, JCO 2007). Around 30% of such patients respond to Epo +/- G-CSF with a median duration of two years (Jadersten, Blood 2005). Azacitidine (Aza) has been reported to induce transfusion-independence (TI) in patients with lower-risk MDS (Silverman, JCO 2002; Lyons, JCO 2009; Musto, Cancer 2010). However, this treatment has not been systematically evaluated in patients resistant to Epo +/- G-CSF, neither has the benefit of the combination of Aza and Epo been assessed in a prospective study. **Methods.** This prospective open-label, non-randomized phase II study included 30 consecutive patients with IPSS low- or int-1 risk MDS and a RBC transfusion need of ≥4 units q 8 weeks. Patients were either refractory to full-dose Epo + G-CSF for >8 weeks, or considered ineligible for such treatment according to a previously published predictive model (Hellstrom-Lindberg, Br J Hem 2003). Enrolled patients were treated with Aza 75 mg/m^2/d for five days q 28 days for six cycles. Patients remaining TD after six cycles were treated with another three Aza cycles, with the addition of Erythropoietin 60,000 units/week. Primary endpoint was number of patients achieving TI after six cycles and secondary endpoints were number of patients achieving TI after Aza+Epo, effect on bone marrow morphology, peripheral blood parameters, and safety profile. **Results.** Thirty patients were enrolled from January 2010 until September 2011. See Table 1 for patient characteristics. Eighteen patients were previously treated with Epo+G-CSF whereas remaining patients scored “low” in the predictive model and were thus considered refractory upfront. Median number of transfusion units needed was 7 (4-14) q 8 weeks preceding inclusion. Median platelet and ANC count pre-treatment was 220x10^9 (22-1468) and 2.1x10^9 (0.3-15.1), respectively. Severe thrombocytopenia (<30) and neutropenia (<0.5) was observed in 3 and 3 patients, respectively.Ten patients pre-terminated the study; five due to sustained cytopenia; two due to death (one sudden death and one neutropenic septicemia); two due to patient’s wish and one due to investigators choice. Twenty-nine serious adverse events were reported in 16 patients with infection (n=19) being most common. Nadir values after each cycle of Aza were seen at week 3 for thrombocytes (median 130x10^9) and at week 4 for neutrophils (median 1.2x10^9), respectively.Twenty-four patients were evaluable for treatment with Aza alone and 14 patients for Aza+Epo. TI was achieved in 5 patients (21%) after Aza alone and in one more patient after Aza+Epo. Three of 6 responding patients had a karyotype including trisomy 8. In a multivariate analysis, trisomy 8 was significantly (p=0.01) associated with response.

Table 1. Patient characteristics.

Median (range) Age	69y (55-80)	Significant fibrosis (grade ≥ 2), n	4
Gender (M/F)	21/9	Median (range) cell percentage	70% (20-100)
Median duration of disease	2y (0-20)	Median (range) blast count	2% (0-9)
Median transfusion units q 8 weeks	7 units (4-14)	IPSS, Low / Int-1, n	6/24
WHO classification, n		WPSS, Low / Int / High, n	3 / 14 / 13
RA / RARS / RCUD	1 / 2 / 2	IPSS Cytogenetics Favorable / Intermed./ Adverse, n	20 / 10 / 0
RCMD / RCMD-RS	13 / 5	Karyotype including trisomi 8, n	4
RAEB-I / MDS 5q-	3 / 1	Median S-epo	443 u/L (25-2313)
MDS/MPD	3	Median S-ferritin	1891 µg/L (220-6230)

Discussion. Aza can induce TI in patients with TD lower-risk MDS, but response rate is lower in this cohort of documented EPO-G-CSF-refractory patients compared to previous reports of less well-controlled cohorts. Since toxicity is substantial, candidate patients for this treatment must be selected carefully. Patients with trisomy 8 show a significantly higher response rate. The combination of Aza and Epo can be effective in rare cases.

0350

TRANSFUSION-DEPENDENT MYELODYSPLASTIC PATIENTS RECEIVING DEFERASIROX: A LONG-TERM FOLLOW UP

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Background. Blood transfusion is the only supportive therapeutic chance in MDS patients refratory to other treatments. Several studies have demonstrated that patients with LOW-RISK MDS (IPSS: LOW, INT-1) have an elevated morbidity and mortality risk after the transfusion of more than 100 units of blood red cells. The use of iron chelators could reduce or prevent the iron overload damage. Deferasirox is a once-daily oral iron chelator that has demonstrated good efficacy and acceptable safety profile. **Aims.** We investigated the effectiveness and the safety of deferasirox therapy in reducing the iron overload in polytrasfused LOW-RISK MDS patients. **Methods.** We have treated 55 patients affected by LOW-RISK MDS refractory to any treatment modality and blood transfusion dependent form at least 1 year. All patients (35 male and 20 female, median age 71 years) showed before the beginning of the iron chelator treatment more than 2000 ng/ml of ferritinemia and a mean blood transfusion request of 1 unit of red blood cells every week in order to maintain Hgb levels higher than 8g/dl. All patients received deferasirox 10mg/kg p.o. once-a-day. A dose escalation to 20 mg/kg and then to 30 mg/kg p.o. once-a-day was performed after respectively one and three months from the beginning of the treatment.Results. After 6 months from the beginning of the therapy with deferasirox all the patients showed a reduction of ferritinemia (an about 60% decrease, r: 58-65%). Interestingly, after 12 months from the beginning of deferasirox therapy, a reduction of the transfusion request (50%) was recorded in 16 patients and one patient was transfusion-independent. To date (48 months of starting therapy) we found a maintenance of efficacy with a persistent reduction of transfusion requirements in about one third of patients. Furthermore, we found no toxicity and/or significant adverse events. **Conclusions.** Our results confirm the effectiveness of the therapy with deferasirox in reducing the iron overload in polytrasfused LOW-RISK MDS patients with acceptable toxicity profile. Moreover, our results show a significant reduction of the transfusion request in about one third of patients, persisting also after 48 months of iron chelation therapy with deferasirox. The positive effect on haemopoiesis of iron-chelation is already known and is due to the reduction of toxicity caused on haemopoietic precursors by an excess of free-iron in the bone marrow. Moreover, recent studies suggest a therapeutic role of deferasirox in MDS, independently of its iron chelating action: deferasirox seems to act as a potent NFkB inhibitor and this property could explain the improvement of the Hgb level.

0351

OUTCOMES OF CONTINUED AZACITIDINE TREATMENT FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME IN IPSS LOW AND INTERMEDIATE-1

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Background. Hypomethylating agents improved clinical outcomes of myelodysplastic syndrome (MDS) patients. However, the treatment duration of azacitidine has not been determined yet, especially in lower risk patients. **Aims.** The current study compared the outcomes of MDS patients with continued azacitidine therapy and those off the treatment in MDS with IPSS low and intermediate-1. **Methods.** A total of 140 MDS patients who treated with azacitidine more than 4 cycles were included in the present study. **Results.** One-hundred six patients were treated with azacitidine 4 to 8 cycles then off the treatment (group-1) and 34 patients were treated more than 8 cycles with median 11 cycles (range 9-40)(group-2). Overall response rate (67.6% vs. 29.2%, p<0.001) and clinical benefit rate (82.4% vs. 53.8%, p=0.003) were higher for group-2 than group-1. Median overall survival (OS; 890 vs. 612 days, p=0.014) and progression free survival (PFS; 659 vs. 398 days, p=0.002) time were significantly longer for group-2 than group-1. Ninety-one patients were IPSS low and intermediate-1 groups. Continued azacitidine therapy improved OS (median 890 vs. 668 days, p=0.063) and PFS (median 685 vs. 433 days, p=0.020) in patients with lower risk IPSS. Survival rates after relapse to azacitidine treatment in lower risk IPSS patients (n=31, 34.1%) were poor and intensive treatment (1-year OS, 54.7%) did not improve the outcome compared to best supportive care (1-year OS, 59.9%; p=0.977). **Conclusions.** Azacitidine improved survival rates in IPSS low and intermediate-1 patients, however, most of the patients progressed with time despite continued azacitidine treatment. Plus, treatment outcomes for patients relapsed to azacitidine were poor despite intensive therapy. So, early implementation of allogeneic transplantation may be incorporated for MDS patients with IPSS low and intermediate-1.

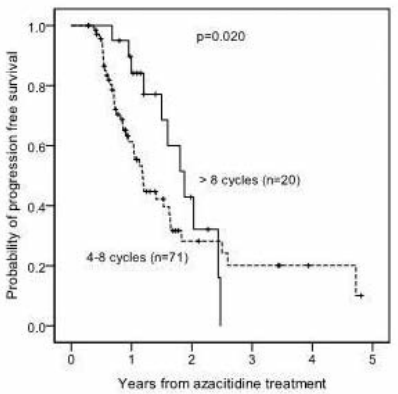

Figure 1. PFS of patients treated with azacitidine more than 4 cycles in IPSS low/intermediate-1 group.

0352

IRON CHELATION THERAPY INDUCES HEMATOLOGICAL RESPONSE IN TRANSFUSION DEPENDENT PATIENT WITH MYELOID MALIGNANCIES

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Background. Iron overload is well known to have a negative impact on survival in chronically transfused MDS patients, that is more striking in lower-risk categories, so that transfusion dependence was included in the WHO Prognostic Scoring System (WPSS) for MDS.Moreover several studies are currently exploring the potential benefits and the mechanisms of action of iron chelation therapies (ICT) on erythropoiesis, due to the increasing number of observations of haematopoietic recovery after ICT in bone marrow failure disorders. **Aims.** We report the results of a retrospective analysis of the hematologic response observed in a cohort of polytransfused patients treated in our institution in the last 10 years, who received ICT. **Methods.** Of 48 unselected polytransfused patients who received ICT, 18 were considered not evaluable, being in treatment with ICT for less than 3 months, ESAs for less than 6 months, 5-Aza for less than 1 year. Of the 30 evaluable patients, 24 were affected by myelodysplastic syndromes (MDS), 5 by myelofibrosis, 1 by severe aplastic anemia. One patient received Deferiprone as ICT, 4 deferoxamine, 28 deferasirox (2 after deferoxamine treatment, 1 before). The median serum ferritin level at the beginning of ICT was 1645 ng/ml (range 1068-6433), the median transfusion requirement was of 3 pRBC units/month (range 2-6); 4 patients also had severe symptomatic thrombocytopenia. **Results.** We observed a cumulative rate of 30% hematologic response (9 of 30 patients), evaluated according to the IWG 2006 criteria as HI-E in 8 patients (1 in deferoxamine and 7 indeferasirox treatment), 5 of whom achieved complete transfusion independency, 1 HI-E+HI-P, 1 HI-P in a patient who, interestingly, did not achieve any erythroid response. The median time to response was 3 months, ranging from 3 to 12, for deferasirox and 3 months for the only patient who experienced HI-E under deferoxamine treatment (following 2 months of previous deferasirox treatment, stopped due to intolerance). The median ferritin level at the time of response was 1760 ng/ml (range 361-2747), showing that the hematological response

was not merely related to the normalization of ferritin the levels.**Conclusion.** In our experience the rate of erythroid response among patients treated with ICT was similar to that reported in literature with standard dose epoetin therapy, in a setting of heavily transfused MDS patients, refractory to standard therapy.Further studies are required to investigate the potential mechanisms of action of iron chelators in inducing hematopoietic response, to determine the clinical and biological features of patients who would gain benefit and to analyze differences between groups of patients showing hematopoietic recovery or not after chelation therapy.

0353

ERYTHROPOIETIN ALPHA VS BIOSIMILAR ERYTHROPOIETIN ALPHA PLUS LIPOFER AND B12 AND FOLATES IN PATIENTS WITH REFRACTORY ANEMIA. TWO CENTER PROSPECTIC STUDY

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Background. Biosimilar drugs have a similar, but usually not inferior although not hidentical effects of old registred drugs. Safety is hidentical to old registred drug**. Aims.** Aim of this study is to verify if in MDS patient with refractory anemia biosimilar erythropoietin alpha is not inferior to erythropoietin alpha in terms of safety, efficacy and costs. **Methods.** Between july 2008 and december 2011, 86 patients affected by refractory anemia were studied. Median follow-up was 18 months (R10-28).Patients were randomized 1:1 to receive in group A erythropoietin alpha 40000 IU sc/weekly.In group B patient received biosimilar erythropoietin alpha 40000 IU sc/weekly. In both arms patients received lipofer 14 mg 2 tablets orally/day calcium levofolinate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group A median age was 70 years (R63-73), M/F: 15/28. In group B median age was 64 years (R60-70), M/F: 24/19. IPSS was low in 30 patients and int-1 in 12 patients, karyotype showed -Y in two patient, del 20q in one patient, trisomy 8 in two patients in group A.IPSS was low in 32 patients and int-1 in 10 patients, karyotype showed -Y in one patients, del 20q in one patient in group B.Patients with 5q- were excluded from this study.Median level of haemoglobin was 9 g/dl in group A (R8-11) and 8.7 g/dL (R8.5-10.5) in group B. Cost of every month of erythropoietinic therapy was calculed dividing for each patient the sum of complete erythropoietic therapy for each month of follow-up, then in each group of patients median cost of erythropoietic therapy was calculed. **Results**. Group A patients increased Hb level of 1 g/dL after

0355

DOES BONE MARROW FIBROSIS AFFECT THE CLINICAL OUTCOME OF PATIENTS WITH MYELODYSPLASTIC SYNDROME OR SECONDARY ACUTE MYELOID LEUKAEMIA TREATED WITH AZACITIDINE?

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Background. Azacitidine improves the overall survival of patients with higher risk myelodysplastic syndrome (MDS) compared with conventional care regimens, but its clinical efficacy is limited in primary myelofibrosis. Bone marrow fibrosis is a common histological feature detected in MDS and confers an adverse prognosis. There is paucity of data about the influence of marrow fibrosis on the clinical effect of azacitidine in MDS. **Aims.** We performed a retrospective analysis in patients with MDS and secondary acute myeloid leukaemia (sAML) with background myelodysplasia or preceding MDS treated with azacitidine. We aim to determine if the degree of marrow fibrosis in MDS would influence the clinical outcome after azacitidine treatment. **Methods.** Total 27 patients who had MDS (n=18) and sAML (n=9) received azacitidine (administered subcutaneously at 75mg/m^2 daily for 7 days with repeat cycle every 4 weeks) with or without oral valproic acid (50mg/kg in 3 divided doses per day for 7 days, given concurrently with azacitidine) from December 2009 to December 2011 in our institution. The clinical, haematologic and cytogenetic data were collected. The bone marrow fibrosis grading system was adopted from Bauermeister (1971). Clinical response and survival outcome were compared between patients with marrow fibrosis less than grade 2 (marrow fibrosis<2) and those with marrow fibrosis of grade 2 to grade 4 (marrow fibrosis≥2). Statistical analysis was performed using SPSS version 17.0. **Results.** Sixteen patients had marrow fibrosis<2 and 11 patients had marrow fibrosis≥2. The median age of the whole cohort (n=27) was 67 years (range 41-82; median age of 68 years for marrow fibrosis<2 and 65 years for marrow fibrosis≥2). Seventeen patients were older than 65 years at the time of first treatment cycle. Cytogenetic risk categories were favourable (n=8), intermediate (n=10) and high (n=9). Among the high-risk cytogenetic category, 7 patients had marrow fibrosis≥2 and 2 patients had marrow fibrosis<2. A correlation of the cytogenetic risk categories and the degree of marrow fibrosis was observed in our cohort (p=0.016). The median numbers of treatment cycles delivered were 3 for marrow fibrosis<2 and 4 for marrow fibrosis≥2. Overall response rates were similar with 44% for marrow fibrosis<2 and 45% for marrow fibrosis≥2 (p=0.93). Age, gender and cytogenetic risk category had no impact on the overall response rate. The median overall survival was 10 months for the whole cohort. The median survival for marrow fibrosis<2 was 19 months and for marrow fibrosis≥2 was 10 months, but the difference in survival was not statistically significant (p=0.465). Further analysis of our cohort of patients showed an improved median survival in patients who had favourable cytogenetic risk (p=0.042), received at least 3 cycles of azacitidine (p<0.001) and/or achieved overall response to treatment (p=0.021). **Conclusions.** This retrospective analysis indicates that bone marrow fibrosis does not impact on the overall response and survival outcome in our small series of patients with MDS or sAML with background myelodysplasia treated with azacitidine. Study of a larger cohort of MDS patients treated with azacitidine is necessary to confirm this finding.

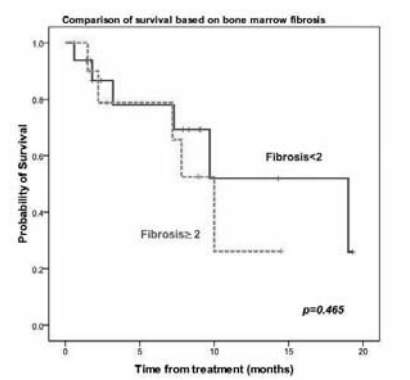

Figure 1. Survival based on bone marrow fibrosis.

0356

CONGENITAL BONE MARROW FAILURE SYNDROMES - DISEASES NOT EXCLUSIVE OF CHILDREN

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Background. The congenital bone marrow failure syndromes (BMFc) are a heterogeneous group of diseases characterized by a defect in hematopoyesis, that may affect one or more blood lineages, and a high oncogenic susceptibility. In the last decades many of the genetic abnormalities implied in these diseases had been clarified, allowing a better understanding of some of the mechanisms involved in normal hematopoyesis. Many patients with BMFc have typical phenotypic abnormalities and the majority is diagnosed in childhood. However, the phenotypes severities are heterogeneous and a growing number of patients are being diagnosed in adulthood. **Aims.** Analyze the clinical and laboratory findings at diagnosis, the evolution to bone marrow failure or to hematological malignancies and survival of patients with BMFc diagnosed in our department. **Methods.** Retrospective and descriptive study of clinical records of patients diagnosed with BMFc between 1996 and 2010. Collected data: sex, age, phenotypic abnormalities and cytopenias at diagnosis, family history, screening tests, clinical evolution, with or without allogenic stem cell transplantation, and survival. **Results.** Among 23 patients with BMFc diagnosed in a 15 years period, we found 8 Fanconi anemias (FA), 6 Blackfan Diamond syndromes (BD), 3 Shwachman-Diamond syndromes (SD), 2 Absent Radius Thrombocytopenia syndromes (ART), 1 Congenital amegacaryocytic thrombocytopenia (amega.T) and 3 osteopetrosis. The median age at diagnosis was 4.5 years; one female was diagnosed with BD at the age of 50 years, at the same time of her 10 years son, following an investigation of a macrocitic anemia. Eleven patients showed cytopenias at presentation, 13 were studied due to phenotypic abnormalities or positive family history. Eight patients with FA evolved to marrow failure, 2 of them to acute myeloblastic leukemia (AML) and 5 were submitted to allogenic stem cell transplantation. Of the 15 remaining BMFc, 4 evolved to marrow aplasia and only 3 patients were transplanted. In this group of 23 patients, 3 died, all with FA.The results are summarized in Table 1. **Summary.** All patients with FA evolved to bone marrow failure, 2 of them to AML, which is consistent with the literature. We did not observe non-hematological neoplasms. Eight patients were submitted to allogenic stem cell trans-

plantation, 5 with FA, 1 with BD, 1 with amega.T and 1 with osteopetrosis. This approach is an imperative attempt to increase patients's survival. BMFc are traditionally underdiagnosed and its identification remains a challenge mainly because their phenotypic characteristics and severity are heterogeneous. BMFc are known as children's diseases but as we can see in this study, some patients have been diagnosed in the adulthood. This is also true because they are expected to have multiple phenotypic and hematological abnormalities, which we show it is not always verified.

Table 1. Clinical data and evolution.

		Fanconi A.	B. Diamond	Shwachman	R.A.T.	Amega.T	Osteopetrosis
N° Cases		8	6	3	2	1	3
M:F		4:4	2:1	2:1	0:2	0:1	2:1
At Diagnosis	Median Age (years)	6	2	12	0	1	2
	Age (limits)	0,1 - 12	0,3 - 50	1 - 4	0	1	0,5 - 4
	Familiar History	4/8	1/6	2/3	1/2	-	0/3
	Screnning Test (+)	8/8	2/6	3/3	-	1/1	-
	Morfologic Abnormalities	5/8	-	-	2/2	-	-
	Cytopenias	3/8	6/6	2/3	2/2	1/1	3/3
Evolucion	Marrow Failure	8/8	1/6	1/3	-	1/1	1/3
	Hematological Neoplasias	2/8	-	-	-	-	-
	Allogenic Transplantation	5/8	1/6	-	-	1/1	1/3
	Death	3/8	-	-	-	-	-

0357

MONITORING OF MORPHOLOGICAL FEATURES OF HEMATOPOIESIS IN MDS PATIENTS UNDERGOING EPIGENETIC THERAPY

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Background/introduction: Treatment with inhibitors of DNA-methylases restores expression of cellular oncosupressor genes in MDS cells and drives them into differentiation to the mature cellular elements. However, therapy with hypomethylating agents may cause severe myelosuppression.**Purpose:** to study morphological features of hematopoiesis in bone marrow aspirates and trephine biopsies of MDS patients taken at different time points after decitabine therapy.**Materials and Methods.** 75 bone marrow aspirates and 67 trephine biopsies from 13 high risk MDS patients have been analyzed before and after 2, 4 and 8 decitabine courses of therapy. **Results.** Complete remission was achieved in 6/13 (46 %) of patients. In most cases decitabine treatment lead to a hypoplasia, characterized by the reduction of granulocyte lineage at the first stages of treatment and its subsequent restoration observed in the patients who have reached remission. In 4/6 (66,7%) of CR patients small prevalence of erythroid lineage persisted as detected by threphine biopsy and became normal - by cytology. The megakaryocytic lineage in 11/12 (92 %) of patients was expanded before treatment and was narrowed to normal or below normal level after therapy in 75 % of patients. Signs of myelodysplasia decreased in complete remission or disappeared in all cell lineages in the majority of patients. **Conclusions.** If CR is achieved after decitabine, almost all morphological features of dysplasia disappears. Cumulative analysis of bone marrow aspirates and trephine biopsies is required to objectively estimate morphological features of hematopoiesis in these patients.

Table 1. Dynamics of hematopoietic lineages while decitabine therapy.

Hematopoietic lineages Bone marrow aspirates	before n=13	after 2 courses n=13	after 4 courses n=7	after 8 courses n=4
Granulocyte cells (normal values 50-70%)	29,3% (16,0-49,6)	17,9% (3-32,6)	34,4% (5,0-50,4)	45,7% (36,5-58,0)
Erythroid cells (normal values 20-30%)	28,5% (2,0-74,5)	33,0% (4,0-67,4)	20,4% (1,0-37,8)	19,6% (8,8-35,5)

0358

CLINICAL EXPERIENCE WITH AZACITIDINE IN CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) BASED ON 27 PATIENTS FROM A SPANISH STUDY

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Background. Chronic myelomonocytic leukemia (CMML) is a clonal disorder of hematopoietic stem cells often occurring in elderly patients (Greco et al. Mediterr J Hematol Infect Dis. 2011). According to the 2008 WHO classification, CMML is a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) further subdivided according to % of blasts in peripheral blood and bone marrow (BM) as CMML-1 (<5% blasts in peripheral blood and 5-9% blasts in BM) and CMML-2 (<10% blasts in peripheral blood and 10-19% blasts in BM) (Vardiman et al. Blood. 2009)Azacitidine (AZA) is a hypomethylating agent approved in Europe for treatment of myelodysplastic syndromes (MDS) with an intermediate to high risk of progressing to acute myeloid leukemia (AML) or death, CMML and AML that has developed from a myelodysplastic syndrome (Prescribing Information EMA 2011). Until its approval in May 2009, AZA was used in Spain under compassionate use in clinical trials.AZA produces a direct decrease of DNA methyltransferase activity *in vitro* and *in vivo*, reverting aberrant DNA methylation and increasing the expression of silenced genes, leading to cellular differentiation and/or apoptosis (Greco et al. Mediterr J Hematol Infect Dis. 2011). **Aims.** To assess the effectiveness of AZA in the treatment of CMML. **Methods.** We report the results (Response, Overall Response, Overall Survival and Progression Free Survival) of a retrospective, longitudinal, multicenter Spanish study of 27 patients: **Results.** Eighteen (66.6%) patients had CMML-1 and 8 (29.6%) had CMML-2. Median age at diagnosis was 69 years. Male/female ratio was 19/8 and ECOG performance status was 1-2 in 96.3% of patients. AZA was initiated at 75 mg/m^2 in 21 patients (78%) and 50 mg/m^2 in 5 (18.5%) patients. The mean number of cycles received was 8.3 (95% CI - 5.91; 10.73). Among the 23 patients evaluable for response, overall response was 39.1% consisting of: 13 % complete response (CR), 13 % partial response (PR), 4 % complete marrow response (mCR) and 8.7% hematological improvement (HI). Stable disease (SD) was noted in 26% and 34.8% had progressive disease (PD). 33.3% patients were alive at the end of treatment with AZA. Median overall survival was 11.70 months (95% IC 7.80, upper limit not reached) (Figure 1). **Conclusions.** Our results show that AZA is an active drug in the treatment of patients with CMML, with similar response rates described in the published literature. These findings corroborate clinical findings from other studies as AZA-001.tatumgonzalez@hotmail.com

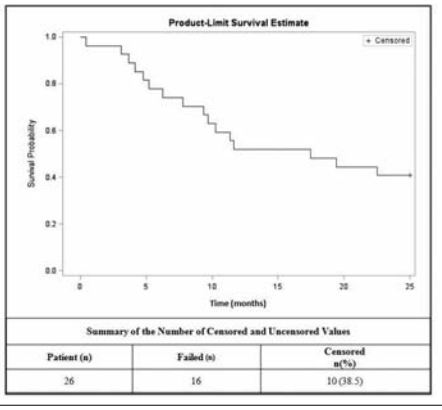

Figure 1. Overal survival.

0359

THE IMPACT OF IRON CHELATION THERAPY ON CLINICAL OUTCOMES IN REAL-WORLD LOWER-RISK PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS): RESULTS FROM THE DÜSSELDORF REGISTRY

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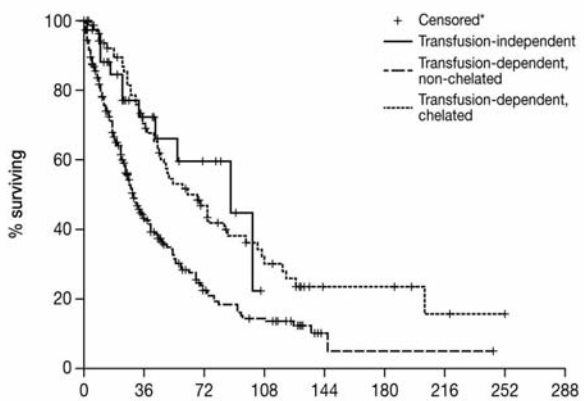
Background. Over time, complications of chronic transfusions and subsequent iron overload can become significant for many patients with MDS, and iron chelation therapy (ICT) will need to be considered. Retrospective evidence of the benefit of ICT on MDS patient clinical outcomes has been previously reported (Fox *et al. Blood* 2009; Rose *et al. Leuk Res* 2010). Established in 1982, the Düsseldorf registry is the largest European database containing information on patients with MDS. Data collected allow examination of the impact of transfusion and ICT practices on long-term clinical outcomes in patients with MDS. **Aims.** This was a retrospective survey of the Düsseldorf registry to assess the impact of transfusion and ICT on clinical outcomes in real-world lower-risk MDS patients. **Methods.** Data were collected from patients with lower-risk MDS diagnosed since 1990, with serum ferritin measurements, who were not chelated or who had received deferasirox and/or deferoxamine (DFO). Patients were considered transfusion-independent or transfusion-dependent, based on their transfusion requirements at or after diagnosis (>2 units of red blood cells/8 weeks). Transfusion-dependent patients were considered to be chelated if they received ≥6 months of chelation therapy, cumulatively. Transfusion-dependent patients were subsequently assigned to either chelated or non-chelated groups, and compared to transfusion-independent patients for the purposes of this analysis. Data were evaluated up to 30 June 2011, corresponding to date of last follow-up. Kaplan-Meier estimates were used to calculate overall survival from time of MDS diagnosis. **Results.** Data from 417 patients were analyzed in the three groups: transfusion-independent patients (n=43; mean [SD] age, 66.0 [11.5] years; 79.1% males), and transfusion-dependent, non-chelated (n=289; 68.9 [12.4] years; 52.2% males) and chelated patients (n=85; 62.7 [10.3] years; 49.4% males). Overall 28 patients received deferasirox; 43 received DFO; 14 received deferasirox and DFO. Data on comorbidities were collected only at patient entry; cardiac (34.9, 23.2, 22.4%), hepatic (16.3, 4.2, 7.1%) and renal (7.0, 11.8, 8.2%) conditions were present among transfusion-independent, non-chelated and chelated patients, respectively.By study cut-off, 27.9% of transfusion-independent patients had died (median [25th, 75th percentile] time to death, 88 months [33, 101]). Among transfusion-dependent patients, 62.3% of non-chelated and 60.0% of chelated patients had died. In non-chelated patients, time to death was 30.0 months (13, 68), compared with 67 months (33, 126) in chelated patients (Figure 1). Overall, 4.7% of transfusion-independent patients progressed to acute myeloid leukemia (AML). Among transfusion-dependent patients, 16.6% of non-chelated patients and 14.1% of chelated patients had progressed.



Patients, n									
Transfusion-independent	43	15	7	0	0	0	0	0	0
Transfusion-dependent, non-chelated	289	81	29	17	2	1	1	0	0
Transfusion-dependent, chelated	85	52	28	16	5	5	2	1	0

*All patients who did not die are censored. If a patient is ongoing in the registry, the patient was censored at the cut-off date.

Figure 1. Kaplan-Meier survival curve for patients with lower-risk MDS from the Düsseldorf registry.

Summary and Conclusions. Transfusion-independent patients with lower-risk MDS had better overall survival and lower AML progression than transfusion-dependent patients. Among transfusion-dependent patients, overall survival was higher in chelated patients than non-chelated patients. Patients were selected based on knowledge of their transfusion need and availability of serum ferritin measurements; transfusion-independent patients were difficult to identify, hence the low numbers. The impact of comorbidities during follow-up could not be assessed since data were collected at entry only. The benefit of ICT on clinical outcomes needs confirmation with well-designed prospective trials.

0360

LONG-TERM OUTCOME OF ACQUIRED APLASTIC ANEMIA: COMPARISON BETWEEN BONE MARROW TRANSPLANTATION AND IMMUNOSUPPRESSIVE THERAPY

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Background. Both immunosuppressive therapy (IST) and bone marrow transplantation (BMT) are accepted treatments for patients (pts) with aplastic anemia (AA). Choosing one of these therapies depends on many factors. The aim of this retrospective study was to compare survival rates and long-term complications after IST or BMT in pts with AA and to identify prognostic factors associated with improved survival. **Patients and Methods.** between 2/1986 and 2/2012 55 pts with newly diagnosed AA were treated either with allogeneic BMT (20 pts) or with IST (35 pts). The median time from diagnosis to therapy was 2 months (range 1 to 17) in IST and 2.5 months (range 1 to 16) in BMT group. There was no statistical difference between 2 treatment groups in sex, severity of AA, disease duration, previous transfusion support, except age (pts in BMT group were younger). Twenty three allogeneic BMT were performed in 20 pts. All donors were HLA-identical sibling (1 donor was identical twin). Source of stem cells was bone marrow in 17 (3 with second transplants) and peripheral stem cell in 3 BMTs. Conditioning regimens were based on cyclophosphamide (CY) with antithymocyte (ATG) in 17 and Flud with CY and ATG in 3 BMTs. Twenty two pts received combined IST with ATG or ALG (antilymphocyte globuline), cyclosporine A and steroids and 13 pts ATG with steroids (from which 5 were splenectomized). **Results.** engraftment was documented (median time 19 days) in 19 transplanted pts (95%). In the first 100 days died 3 (15%) transplanted pts: one without engraftment (5%) on +23 days with gram-negative sepsis, the other developed steroid-refractory aGvHD grade 3-4 with invasive fungal infection and died on 78 days and the third died on 60 days with pneumonitis interstitialis (CMV+). Three pts (15.7%) who rejected allograft after 7 months (range 6 to 9), were retransplanted: 2 successfully, 1 died 8 days following the second BMT. One female pt (5.2%) developed cGvHD. In IST group responded 29 pts (82.8%) and 7 of them (24%) had two cycles IST. Five pts (14.2%) from IST group died, major causes of death were infection and hemorrhage. Aplasia recurred in 6 pts (20.6%) with partial response following IST in 24 months. Long-term complications of IST were: an evolution in MDS/AML after 76 months at 2 pts (6.8%), in PNH after 80 months at 1 (3.4%), avascular necrosis of hip at 5 pts (17.2%) and Ca prostate gland (after 150 months) at 1 pt (3.4%). Between groups did not difference in probability 12-years overall survival (BMT group 59.2%±0.12, IST group 73.4±0.08). Difference in survival was found in pts with/without response/engraftment ($p<0.001$). An improvement of peripheral blood counts was faster following BMT (median 19 days) related to IST (median 4 months) ($p<0.001$). **Conclusions.** This study indicates that over 60% of pts with AA can be successfully treated with either BMT or IST. Long-term overall survival does not differ in the two treatment groups. Questions still remains.

Myeloproliferative neoplasms - Clinical

0361

LONG-TERM FOLLOW UP OF A PHASE 1/2 STUDY OF SAR302503, AN ORAL JAK2 SELECTIVE INHIBITOR, IN PATIENTS WITH MYELOFIBROSIS (MF)

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Background. SAR302503 is a potent, oral, JAK2-selective inhibitor being studied for the treatment of high- or intermediate-risk primary, post-polycythemia vera (PV), or post-essential thrombocythemia (ET) MF. We conducted a dose-escalation study (NCT00631462) followed by an extension study in patients who continued treatment beyond 6 cycles (NCT00724334) to evaluate the long-term effects of orally administered SAR302503 in MF (primary, post-PV, post-ET). Interim safety and efficacy results have been published (JCO 2011;29:789-796). **Aims.** The aim of this analysis is to report updated safety and efficacy data from the cohort remaining on study, including an assessment of the JAK2V617F allele burden. **Methods:** SAR302503 was administered orally once daily in 28-day cycles. Eligibility criteria included age ≥18 years and a platelet count ≥50x10^9/L. Patients were allowed into the extension study if treatment was deemed beneficial and well tolerated. The assigned dose of SAR302503 was the same as the last received in the dose escalation/confirmation study, but not to exceed the maximum-tolerated dose (MTD). All patients provided written informed consent. **Results.** Patients (N=59) were enrolled between February and October 2009: 44 had primary MF, 12 post-PV MF, and 3 had post-ET MF. The median age was 64 years, 51/59 patients (86%) were JAK2V617F-positive, and the median palpable spleen size was 18 cm at enrollment. Twenty-eight out of 59 patients (47%) were treated in the dose-escalation cohort (30-800 mg), 31/59 (53%) were treated at the MTD (680 mg), and 43/59 (73%) completed 6 cycles and continued on the extension study. As of June 30, 2011, 23/59 patients (39%) remained on treatment; the median number of cycles was 30 (range, 13-44), and the median current dose was 440 mg. As previously reported, spleen responses were seen within the first 3 cycles, with ≥50% patients in each dose level ≥240 mg/day showing a durable ≥50% decrease in palpable spleen size. The proportion of patients with a ≥50% reduction in spleen size was 54% at 6 months (n=57); 67% at 12 months (n=42); 53% at 18 months (n=36); 55% at 24 months (n=31); and 61% at 30 months (n=18). After 24 cycles of treatment, patients who had a JAK2V617F allele burden >20% at study entry had a persistent decrease in the median percentage JAK2V617F allele burden compared with baseline (21% [n=12] vs 60% [n=23]; P=0.03). Of the 34 patients who had leukocytosis at baseline, about 50% achieved a normalization of white blood cell counts across cycles. Of the 11 patients, who had thrombocytosis at baseline, platelet counts normalized in up to 86% of patients over time. Treatment-emergent toxicities were dose-dependent and generally alleviated with dose-reduction. Most frequently reported adverse events included nausea, diarrhea, vomiting, anemia, and thrombocytopenia. **Summary and Conclusions.** An updated interim analysis of this phase 1/2 study shows that treatment with SAR302503 reduced spleen size and the JAK2V617F allele burden, with an acceptable safety profile in patients with primary MF, post-PV MF, or post-ET MF. A phase 3 trial of SAR302503 in this setting was initiated in December 2011 (JAKARTA, NCT01437787). Sponsored by Sanofi.

0362

BURDEN OF ILLNESS OF AGGRESSIVE SYSTEMIC MASTOCYTOSIS (ASM) IN THE UNITED STATES

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Background. Per the 2008 WHO report on the spectrum of mast cell disease, ASM is a severe and debilitating subtype of systemic mastocytosis (SM); mast cell leukemia is an aggressive variant of the disease. Limited information has been published on the epidemiology and burden of ASM. **Aims.** This study reviewed the epidemiologic, clinical, humanistic and economic literature on ASM in order to estimate the burden of ASM in the US. **Methods.** A systematic literature review was conducted in PubMed to identify English language publications regarding ASM published 2000 - 2011. 181 citations were identified, 156 abstracts screened, and 6 articles abstracted. The US population-level burden of ASM was estimated using an Excel model. Direct treatment costs were calculated from treatment patterns described in identified publications. **Results.** ASM involves multiple organ systems, resulting in potentially severe symptoms/conditions including anaphylaxis/allergic reactions, osteoporosis, hepatomegaly, splenomegaly, gastrointestinal symptoms, fatigue, and weight loss. There is no known cure for ASM. Treatment focuses on symptom reduction with antihistamines, bisphosphonates, glucocorticoids, proton pump inhibitors and mast cell stabilizers. Medications to control the disease include interferon-alpha, cladribine (2CdA), hydroxyurea, and imatinib or investigational agents in clinical trials. Median survival was 41 months in a published cohort study.No publications were identified providing US epidemiology data. Two estimates were calculated for the prevalence of ASM in the US in 2010: 616 and 1,220. A global ASM prevalence estimate of 0.2 cases/100,000 individuals resulted in an estimate of 616 prevalent cases. An SM prevalence rate of 3.3 cases/100,000 individuals and assumption that ASM accounts for 12% of SM cases resulted in a US prevalence rate of 0.4 cases/100,000 individuals and an estimated 1,220 ASM cases. Incidence was estimated at only 111 cases in 2010 using an SM incidence rate of 0.3/100,000 (again assuming ASM accounts for 12% of SM cases). The proportion of ASM/SM cases ranged from 7-18% in the literature with 12% from the largest study.Two studies examining the burden of mastocytosis on patient quality of life or productivity were identified. In one study, 82% of SM patients stated they suffer from a disability, with 28% reporting it as severe or intolerable. A recently published study reported 64% of mastocytosis patients commented that the disease moderately to severely restricted their quality of life. No studies were identified examining the economic burden of ASM. In this model, direct monthly per-patient costs were estimated between $5,232 and $8,741. These are likely underestimated as a result of limited resource utilization information in the literature. **Conclusions.** These results provide preliminary estimates for the burden of ASM in the US. Additional research can assist in further quantifying these estimates. Due to the variety of symptoms experienced by ASM patients, the lack of a curative therapy, impact on quality of life and short survival, it is likely that patients with ASM experience indirect costs (e.g., limitations in functioning or productivity) that may exceed the direct costs of treatment; further evaluation is warranted to estimate the total economic burden.

0363

THE COST OF BLOOD TRANSFUSION IN LOW-RISK PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS): AN ECONOMIC ANALYSIS FOR THE CASE OF GREECE

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Background. Myelodysplastic Syndromes (MDS) represent a heterogeneous group of acquired clonal bone marrow disorders with a diverse spectrum of presentations. Frequently, anemia represents the principal underlying cause of symptoms for MDS. In accordance with the National Comprehensive Cancer Network (NCCN) as well as European guidelines, the National guidelines in Greece recommend the use in selected patients groups of Red Blood Cell (RBC) transfusion for the management of anaemia in MDS patients. Nonetheless, the numbers of blood units donated in Greece by volunteer donors are insufficient to cover the existing needs. In addition, this treatment option carries risks and it also represents a costly option, especially for those who need long-term care, such as patients belonging to the low risk group. **Aims.** Given that healthcare resources are scare, especially in the presence of a serious economic crisis, an economic analysis was undertaken to assess the annual cost per patient of RBC transfusion in low risk MDS patients from the National Health System (NHS) perspective. **Methods.** A micro-costing bottom up approach was used based on a structured questionnaire filled by clinicians. Annual cost accounts for the cost of Blood Collection and Testing (BCAT), the cost of transfusion (CT) and the cost of the iron chelation (IC). Due to lack of robust local data, the cost of adverse events was not considered. The cost of BCAT (per two units) includes the cost of transfusion bag, molecular testing for HIV and hepatitis B/C, the cost of blood separation, group typing cost and storage cost. For the preparation of blood, two hours work by technicians (nurses-biologists) was considered, according to expert advice. The cost of CT per patient includes: the cost of cross-matching, the cost of white-blood-cell removal filters and one day admission in outpatient department (approximately six hours). The cost of

IC is expected in all patients after 25-30 blood transfusions. To deal with uncertainty, bias-corrected uncertainty intervals (UI) were calculated using the percentile method of non-parametric bootstrapping. Drug prices and the unit cost of healthcare resources were taken from local sources. **Results.** The average cost of RBC transfusion per patient year was estimated at €21,759 (95%UI: 20,768 - 22,758). The annual cost of CT was estimated at €2,589 (95%UI: €2,500 -€2,678), while the cost of BCAT was limited to €311 (95%UI: €264-€359). The cost of IC was estimated at €1,977 (95%UI: €1,948 -€2,008) per month and accounts for 86% of total cost. Finally, the cost of transfusing 2 units of blood was estimated at €906 (95%UI: €846-€948). **Conclusions.** Despite the fact that RBC transfusion represents the cornerstone treatment for MDS patients, the availability of blood as well as the cost related to RBC transfusion remain major issues. According to our analysis, the cost of regular blood transfusion in low risk MDS patients is substantial in Greece. This cost in reality could be even higher if the cost of adverse events was also considered. In addition, further investigation is needed in regards to the impact of poor compliance for IC therapy.

Table 1. Cost per item used in the analysis.

Blood collection and testing (2 units)	Cost (€)
Transfusion bag	5
Molecular testing for HIV/ hep B/C	5
Cost of separation	3
Cost of blood group typing	1
2hours of nurse/technician	20
Blood storage cost	3
Cost of blood transfusion (2 units)	
Cross-matching	3
WBC Removal Filter/ vein catheter	2.8
1 day admission cost	280
Cost of Iron chelation therapy (per month)*	
Desferrioxamine**	€0.048 per mg

**This type of cost was calculated only for those patients who need Iron Chelation after 20-30 units of Blood Transfusion*
***Mean dose was estimated as: 20mcg/per kg per day for an adult (70 kilograms)*

0364

A PHASE 1B, DOSE-FINDING STUDY OF RUXOLITINIB PLUS PANOBINOSTAT IN PATIENTS WITH PRIMARY MYELOFIBROSIS (MF), POST-POLYCYTHEMIA VERA MF (PPV-MF), OR POST-ESSENTIAL (PET-MF) THROMBOCYTHEMIA MF

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Background. Ruxolitinib is a potent and selective oral JAK1/2 inhibitor approved in the United States that has demonstrated rapid and durable reductions in splenomegaly and improved disease-related symptoms and quality of life in the 2 phase 3 COMFORT studies. Efficacy may be further enhanced with combinations of agents. Panobinostat, a potent oral pan-deacetylase inhibitor, has shown spleen size reduction, symptomatic improvement, and, in some cases, reversal of marrow fibrosis in MF patients in phase 1/2 trials. Synergistic anti-MF activity was shown in combination with ruxolitinib in JAK2-mutation-driven MF murine models. **Aims.** This study will determine a recommended phase 2 dose (RP2D) and/or maximum tolerated dose (MTD) and evaluate the pharmacokinetics (PK) of ruxolitinib and panobinostat in patients with MF. **Methods.** This is a phase 1b dose-finding study of the combination of ruxolitinib and panobinostat for the treatment of PMF, PPV-MF, and PET-MF (NCT01433445). Patients must be intermediate-1, -2, or high risk by International Prognostic Scoring System (IPSS) criteria and have palpable splenomegaly ≥5 cm below the costal margin. Dose escalation will be guided by a Bayesian logistic regression model with overdose control and will depend on dose-limiting toxicities (DLTs) in the first cycle as well as other safety findings. Each dosing cohort will consist of ≥ 3 evaluable patients. Data for ≥ 9 patients at any given dose level are required to determine the RP2D and/or MTD. Serial blood samples collected following a single dose of ruxolitinib alone on day 1 and in combination with panobinostat on days 2 and 6 were evaluated for plasma concentrations by LC-MS/MS. PK parameters were derived using noncompartmental analysis. **Results.** The first cohort of 5 patients (PMF, n = 2; PET-MF, n = 3) was treated with ruxolitinib 5 mg twice daily (BID) and panobinostat 10 mg 3 times a week, every other week (TIW/QOW); the second cohort of 8 patients (PMF, n = 4; PET-MF, n = 2; PPV-MF, n = 2) was treated with ruxolitinib 10 mg BID and panobinostat 10 mg TIW/QOW. Spleen and symptom responses for patients in the first 2 cohorts are shown (Table 1). In the first cycle, 1 DLT (grade 4 thrombocytopenia) and 1 severe adverse event (grade 3 nausea and diarrhea) were reported, and 2 patients had grade 1 elevations of creatinine. No clinically significant EKG abnormalities have been observed to date. The inter-individual variability in ruxolitinib AUC is ~40%. Mean ruxolitinib AUC on day 1 was comparable to AUC when combined with dose 1 panobinostat on day 2, but increased by 30% following 3 doses of panobinostat on day 6 in cohort 2. **Conclusions.** The combination of ruxolitinib and panobinostat appears to be well-tolerated and synergistic with promising efficacy results. Careful dose titration with additional cohorts will be necessary to confirm the optimal dosing strategy for this promising combination in the treatment of MF patients.

Table 1. Response to the combination of Panobinostat and Ruxolitinib for individual patients.

	Spleen		Constitutional Symptoms	
	Response	Time	Response	Time
Cohort 1 (n = 5)				
Patient 1	50% reduction	End of week 2	Complete resolution	End of week 2
Patient 2	50% reduction	End of week 2	Complete resolution	End of week 2
Patient 3	42% reduction	Start of cycle 4	No change	Start of cycle 4
Patient 4	17% reduction	Start of cycle 3	No change	Start of cycle 4
Patient 5[a]	No change	Start of cycle 4	No change	Start of cycle 4
Cohort 2 (n = 8)				
Patient 1	83% reduction	During week 1	Complete resolution	After 48 hours
Patient 2	30% reduction	After week 2	Complete resolution	After 72 hours
Patient 3	20% reduction	End of week 2	Significant improvement	End of week 2
Patient 4	20% reduction	End of week 2	Significant improvement	End of week 1
Patient 5	75% reduction	End of week 2	Complete resolution	End of week 2
Patient 6[b]	50% reduction	End of week 3	No change	End of week 3
Patient 7	No change	End of week 3	Complete resolution	End of week 3
Patient 8	Complete resolution	End of week 3	No change	End of week 3

[a] Patient experienced rising blast counts and was taken off study to undergo stem cell transplantation.

[b] Patient experienced grade 4 thrombocytopenia and was taken off study due to DLT criteria.

0365

GERMLINE ACTIVATING JAK2-MUTATION IN A FAMILY WITH HEREDITARY THROMBOCYTOSIS

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Background. The association between somatic JAK2 mutation and myeloproliferative neoplasms is now well established. However, JAK2 mutations are associated with heterogeneous clinical phenotypes and have been described both as primary and secondary genetic events, making it challenging to understand their exact role in disease pathogenesis. **Aims and Methods.** We have

identified a family with germline JAK2-V617I mutation and have characterised the clinical and biological impact of the mutation **Results.** JAK2-V617I-mutation was detected by pyrosequencing and was present at heterozygous levels in peripheral blood (PB) and germline DNA in six members of the same family. The mutation segregated with hereditary mild/moderate thrombocytosis in a high penetrance, autosomal dominant pattern. Vascular events were frequent in older affected individuals. Bone marrow (BM) examination demonstrated megakaryocyte hyperplasia but no evidence of myelofibrotic or leukaemic transformation.Whole exome sequencing (one case) and screening for MPN-associated mutations by targeted sequencing (four cases) did not reveal evidence of additional genetic events. Furthermore, copy number abnormalities or areas of uniparental disomy were excluded by SNP array in four cases. In addition, we carried out XCIP clonality analysis on two potentially informative cases with no evidence of clonal haematopoiesis.Previous studies have suggested that the JAK2-V617I mutation is constitutively activating in retrovirally transduced cell lines (PMID:18326042). However, patients with heterozygous JAK2-V617I did not show cytokine-independent colonies, suggesting that the impact of JAK2-V617I on signalling might be distinct from V617F. Indeed, in autonomous EpoR-Ba/F3 JAK2-V617I cells, constitutive STAT3 and STAT5 activation was much weaker for JAK2-V617I than V617F, and no activation of STAT1 was observed. In response to Epo stimulation, however, V617I resulted in markedly increased downstream signalling in comparison with wild-type JAK2 and comparable to JAK2-V617F. In order to confirm aberrant signalling in the JAK2-V617I patients, we investigated pSTAT3 levels in their PB cells. Baseline pSTAT3 level was not altered, however, following GCSF stimulation, JAK2-V617I-positive PB CD33+ myeloid and CD34+ stem/progenitor cells demonstrated a marked increase in pSTAT3 levels in comparison with controls.Phenotypic haematopoietic stem cells (Lin-CD34+CD38-CD90+CD45RA-) were increased in the PB and BM of JAK2-V617I positive patients and CD34+ cells showed normal engraftment in xenotransplantation assays. This is particularly noteworthy as the impact of JAK2 mutations on stem cells in mouse models is controversial. There were no significant differences in numbers of other phenotypically defined progenitor populations. In comparison with controls, CFU-GM were increased in the PB and BM of V617I-positive cases but BFU-Es were not affected, and CFU-Mks were non-significantly increased. **Summary and Conclusions.** Germline JAK2-mutation is a previously unreported cause of inherited haematopoietic disease. The high penetrance and homogeneous phenotype, together with cytokine hyper-responsive JAK2-dependent signaling in the presence of JAK2-V617I, provides compelling evidence of the causative nature of the mutation. Our findings highlight the need to routinely screen for non-JAK2-V617F mutations, and to exclude germline mutation in selected cases. Whilst these are likely to be rare families, we predict that other germline JAK2 mutations will now be detected, and genotype-phenotype correlations will be highly informative for understanding of JAK2-mutation biology.

0366

CONVENTIONAL THERAPEUTIC OPTIONS HAVE LIMITED IMPACT ON MPN SYMPTOMS: INSIGHTS FROM A PROSPECTIVE ANALYSIS OF THE MPN-SAF TSS

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Background. Symptom burden in MPNs is severe and a risk factor for mortality in some disease subtypes (Blood 2010;115(9):1703-8). A recent trial comparing JAK2 treatment to best available therapy revealed that patients receiving conventional MPN therapies experienced no difference or worsening of symptoms (Blood 2011;118(21):a6501). No studies have evaluated the specific associations of conventional therapies on individual MPN symptoms using a validated patient-reported measure of symptom burden. **Aims.** We aimed to assess associations between conventional therapies on specific MPN symptoms using the MPN-SAF TSS (Blood 2011;118(21):a3839). **Methods.** Patient demographics, symptom burden and disease traits including therapies were collected from MPN patients and physicians at a single time point during therapy. MPN-SAF TSS included "worst"fatigue from the BFI and MPN-SAF items of concentration, early satiety, inactivity, night sweats, itching, bone pain, abdominal discomfort, weight loss and fever. MPN-SAF TSS was calculated as the total 10-item score, reported in a range of 0 to 100 for patients completing at least 6 of the 10 items. **Results.** *Demographics*: 1433 MPN patients were prospectively enrolled, including 594 essential thrombocythemia(ET), 538 polycythemia vera(PV) and 293 myelofibrosis(MF) patients from 11 countries. Of these, 1408 patients completed at least 6 of the 10 required items. Patients were 54% female with a median age of 63.*Therapies:* Common therapies included hydroxyurea/hydroxycarbamide(47.7%), salicylates(43.8%), interferon/pegylated-interferon(9.4%), and phlebotomies(5.3%) with 12.6% receiving no therapy. MPN-SAF TSS variations were observed between patients receiving and not receiving each therapy, although only one reached statistical significance (Table 1).

Table 1. Number of respondents and average MPN-SAF TSS Score by type of MPN treatment (N=1,408).

			MPN Treatment							
	Any Treatment		Hydroxyurea/ Hydroxycarbamide		Aspirin		Interferon/pegylated-interferon		Phlebotomies/ Venesection*	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
ET										
N	494	90	287	297	300	284	36	548	--	--
MPN-SAF TSS Average	*18.6*	*18.9*	*18.5*	*18.8*	*18.0*	*19.4*	*21.3*	*18.5*		
PV										
N	499	30	274	255	260	269	69	460	73	456
MPN-SAF TSS Average	*22.1*	*16.8*	*20.7*	*23.0*	*22.3*	*21.3*	*25.5***	*21.2***	*20.6*	*22.0*
MF										
N	232	57	104	185	59	230	26	263	--	--
MPN-SAF TSS Average	*24.6*	*28.3*	*24.1*	*26.0*	*22.1*	*26.1*	*25.0*	*25.3*		

*ET and MF not assessed
**Student's t-test indicated a significant difference in MPN-SAF TSS between PV patients who received interferon therapy compared to those who did not (p=0.04).

Overall Treatment Effects: When comparing the overall effects of current therapy, worst fatigue was significantly more severe among PV patients undergoing treatment as compared to patients not receiving treatment (4.5(n=497) vs. 3.4(n=30), p=0.03). Among MF patients, abdominal comfort was significantly worse among patients not receiving therapy (3.3(n=57) vs. 2.3(n=230),p=0.02) .*Aspirin:* ET patients receiving aspirin reported significantly less weight loss than patients not receiving salicylates (0.62(n=298) vs. 0.98(n=283),p=0.03). Additionally, aspirin among ET patients reduced pruritus (1.5(n=296) vs. 2.0(n=284),p=0.02). PV patients receiving aspirin reported more concentration difficulties than patients on a non-aspirin regimen (3.0(n=254) vs. 2.4(n=264),p=0.01). MF patients receiving aspirin reported decreased bone pain (1.5(n=58) vs. 2.4(n=228),p=0.04) and abdominal discomfort (1.9(n=58) vs. 2.7(n=229),p=0.04). *Hydroxyurea/hydroxycarbamide*: PV patients receiving hydroxyurea/hydroxycarbamide reported decreased itching than patients not on this therapy, although this effect was of borderline significance (2.5(n=272) vs. 3.0(n=255),p=0.05). *Phlebotomies:* PV patients being given phlebotomies had significantly decreased problems with concentration compared to non-phlebotomized patients (1.9(n=71) vs. 2.8(n=447),p=0.02). *Interferon/pegylated-interferon*: PV patients receiving interferon therapy reported increased early satiety (3.1(n=69) vs. 2.4(n=257),p=0.04), fever (0.7(n=67) vs. 0.3(n=455), p=0.02), and MPN-SAF TSS (25.5(n=69) vs. 21.2(n=460),p=0.04) than non-interferon-receiving counterparts. **Conclusions.** Few significant effects in overall symptom burden were observed in patients undergoing traditional MPN therapies. Though some effects reached nominal statistical significance, a portion

may be spurious given the large number of tests performed. Further studies are needed to determine whether treatments impart additional symptom burden or if patients with specific symptom characteristics are selected to receive a particular treatment. Prospective serial assessment of conventional therapy impact is ongoing as part of a new clinical trial.

0367

IMPROVED SURVIVAL IN YOUNGER PATIENTS WITH PRIMARY MYELOFIBROSIS OVER TIME, A STUDY OF 1,048 PATIENTS DIAGNOSED WITH PRIMARY MYELOFIBROSIS IN SWEDEN 1973-2008

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Background. Primary myelofibrosis (PMF) is the subtype associated with the worst prognosis of the myeloproliferative neoplasms (MPNs), overall median survival has been reported to be five to six years. Recent prognostic scoring systems all include age >65 years as an indicator of poor prognosis. We recently reported that there has been no major overall improvement in survival in PMF patients during the last three decades. However there is limited detailed information regarding the impact of age on survival over time. **Aims.** To assess patterns of survival in PMF patients of different age groups in a population-based setting in Sweden during a study period of 36 years. **Methods.** Patients with PMF were identified through the Swedish Cancer Register from 1973-2008 with follow-up until 2009. Relative survival ratios (RSRs) were computed as measures of survival. Five-, 10-, and 15-year RSRs with 95% confidence intervals(CIs) were calculated for PMF patients during four calendar periods:1973-1982, 1983-1992, 1993-2000, and 2001-2008 and for five different age groups; younger than 50, 50-59, 60-69, 70-79, and 80 years and older. Information on the number of allogeneic stem cell transplantations (SCTs) was obtained from the European Group for Blood and Marrow Transplantation Register. **Results.** A total of 1,048 PMF patients were identified (56% males, median age at diagnosis 71 years) were reported to the Swedish Cancer Register 1973-2008. Fifty-eight patients were younger than 50 years at diagnosis. During the study period, 43 allogeneic SCTs were carried out in patients with PMF in Sweden.Survival was lower in PMF patients compared to expected survival in the general population, reflected in 5-, 10- and 15-year RSRs of 0.39 (0.35-0.43), 0.21 (0.18-0.25), and 0.11 (0.08-0.15), respectively. During the first calendar period there was no distinct difference in relative survival between the age groups. However, during the years 1983-2008, patients younger than 50 years at diagnosis had a superior survival compared to the other age groups (Figure 1).

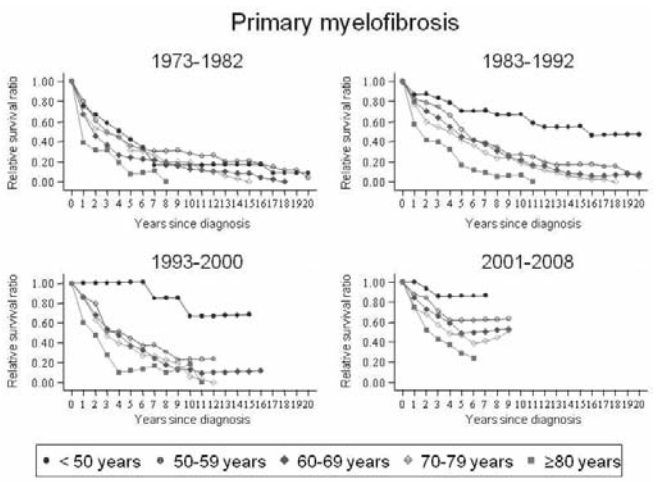

Figure 1. Cumulative relative survival in patients with primary myelofibrosis in relation to age and calendar period at diagnosis.

Five-year survival improved over time in patients <50 years at diagnosis and was 0.42 (0.15-0.67), 0.71 (0.47-0.85), 1.01 (1.01-1.01; no deaths occurred) and 0.86 (0.53-0.97) for the calendar periods 1973-1982, 1983-1992, 1993-2000, and 2001-2008, respectively. Additionally, in the same age group the 10-year RSR also improved and was 0.17 (0.03-0.42), 0.67 (0.44-0.84), and 0.67 (0.18-0.93) in 1973-1982, 1983-1992 and 1993-2000, respectively. **Summary and Conclusions.** In this large population-based study of over 1,000 PMF patients, we found patients under 50 years at diagnosis to have a better survival compared to patients aged 50 or above. Importantly, survival improved in younger PMF patients. There were no major changes in the classification system between 1983 and 2000. However, the introduction of prefibrotic PMF in 2001, including also early stage PMF patients in this group, might have influenced RSRs for the better during the most recent calendar period. In addition, the increasing number of allogeneic SCTs, the decrease in use of leukemogenic drugs and better supportive care has most likely contributed to the improvement in survival in young PMF patients.

0368

A POLYMORPHISM IN THE XPD GENE IS ASSOCIATED WITH HIGHER INCIDENCE OF LEUKEMIC TRANSFORMATION AND NEW NONMYELOID MALIGNANCIES IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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Background. Patients with essential thrombocythemia (ET) and polycythemia vera (PV) have an increased incidence of new hematologic and nonhematologic malignancies as compared to the general population. However, information on the factors determining the risk of such complication is limited. Recently, inherited variations in DNA repair efficiency have been implicated in the predisposition to *de novo* and therapy-related acute myeloid leukemia (AML), as well as in the increased susceptibility to a variety of nonhematologic cancers.

Aims. To evaluate whether constitutional genetic variation in DNA repair predisposes to leukemic transformation and new nonmyeloid neoplasias in patients with ET and PV. **Methods.** Case-control studies for the predisposition to both types of malignancies were nested in a cohort of 422 subjects diagnosed with ET or PV during the period 1973-2010 in several institutions in Spain. A total of 64 incidence cases of AML and 50 of primary nonmyeloid cancers were accrued. Genotyping analysis of five single nucleotide polymorphisms located in four DNA repair genes involved in the nucleotide excision repair (*ERCC2* [also known as *XPD*], *ERCC5* [*XPG*], *XPC*) and base excision repair (*XRCC1*) pathways was performed by real-time PCR. **Results.** By conditional regression analysis, adjusted for other potentially predisposing factors, the Gln/Gln genotype in the *XPD* codon 751 (OR: 4.9; 95% CI: 2.0-12; P=0.001) and exposure to cytoreductive agents (OR: 3.5; 95% CI: 2.0-6.2; P<0.001) were identified as risk factors for leukemic transformation, whereas the Gln/Gln *XPD* genotype (OR: 4.2; 95% CI: 1.5-12; P=0.007) and patients' age (OR: 2.0; 95% CI: 1.4-2.8; P<0.001) predicted for the development of new nonmyeloid malignancies. Presence of the *JAK2* mutation retained a trend to be independently associated with development of a new primary cancer (OR: 2.5; 95% CI: 1.08-5.9; P=0.04) after adjustment for age and the *XPD* genotype. **Conclusions.** The present study shows that a single nucleotide polymorphism in the *XPD* gene predisposes individuals with ET and PV to develop AML and new primary nonmyeloid malignancies. These results, if confirmed in an independent series, could allow the identification of high-risk patients who would benefit from close surveillance and individualized therapeutic approaches.

0369

A PHASE 1B, OPEN-LABEL, DOSE-FINDING STUDY OF RUXOLITINIB IN PATIENTS WITH MYELOFIBROSIS AND BASELINE PLATELET (PLT) COUNTS BETWEEN 50 AND <100 X 10^9/L

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Background. Ruxolitinib, a potent and selective oral JAK1/2 inhibitor, has demonstrated rapid and durable reductions in splenomegaly and improved MF-related symptoms and quality of life in 2 phase 3 studies in patients with PMF, PPV-MF, or PET-MF. There is considerable experience in patients who develop thrombocytopenia on study, and ruxolitinib is well tolerated with dose adjustment. However, there is limited experience in patients with baseline thrombocytopenia as those with low PLTs (<100×10^9/L) were excluded from the phase 3 protocols. **Aims.** The EXPAND (Evaluating RuXolitinib in Patients with Low Baseline PlAtelet CouNts Diagnosed with Myelofibrosis) study is evaluating the safety of ruxolitinib to establish the maximum safe starting dose (MSSD) in thrombocytopenic patients with MF. **Methods.** Phase 1b, open-label, dose-finding study (NCT01317875) in patients with PMF, PPV-MF, or PET-MF and baseline PLTs 50-100x10^9/L. A Bayesian logistic regression model will be used to guide dose-escalation decisions; intrapatient dose escalation is allowed during the study. Study consists of 2 phases: dose-escalation and safety-expansion. Starting dose of ruxolitinib is 5mg twice daily (BID) with a maximum of 15mg BID. In the dose-escalation phase, cohorts will be: 5mg BID, 5mg am/10mg pm, 10mg BID, 10mg am/15mg pm, and 15mg BID; initially patients with PLTs 75-100x10^9/L (first stratum) will be enrolled. Once safety is established at the first 2 dose levels (5 mg BID; 5mg am/10mg pm), patients with PLTs 50-75x10^9/L will be included (second stratum). Each dose level in the second stratum will be open only if both that dose and the following one are deemed safe in the first stratum. In the safety-expansion phase, 20 patients (10 in each stratum) additional to those treated at the MSSD during dose escalation will be treated at the respective MSSD for their stratum. **Results.** Seven patients (PMF, n=4; PPV-MF, n=3) are currently enrolled; 4 in cohort 1 (5 mg am/5 mg pm) and 3 in cohort 2 (5 mg am/10mg pm). At baseline, all patients had an ECOG of 0 or 1, and spleen size ranged from 12-30cm below the costal margin. Six patients completed >28 days of treatment and are consequently evaluable; the seventh patient discontinued at day 6 due to granulocytic sarcoma and was nonevaluable. Of the 6 evaluable patients, 1 patient discontinued treatment on day 57 due to deterioration of general condition and underwent splenectomy on day 64. Reported AEs were similar to those previously seen with ruxolitinib. Three patients experienced grade 3/4 AEs (only 2 anemia events were study-drug related), and 2 patients experienced serious AEs (Table 1).

Table 1. Grade 3 or 4 AEs reported for all patients.

	AE or SAE	Grade	Relationship to Study Drug	SAE	Outcome	Action
Patient 1	Deterioration of general condition	3	Unrelated	No	Recovered/resolved	Discontinued
	Worsening of back pain, radiating to pelvis and hip	3	Unrelated	No	Recovered/resolved	None
	Worsening of neuropathic pain both legs	3	Unrelated	No	Recovered/resolved	None
	Anemia	4	Related	Yes	Recovered/resolved	None
	Splenectomy	4	Unrelated	No	Recovered/resolved	Discontinued
Patient 2	Anemia	3	Related	Yes	Recovered/resolved	None
Patient 3 (nonevaluable)*	Paralysis of oculomotor nerve	3	Unrelated	No	Recovered/resolved with sequelae	Discontinued
	Granulocytic sarcoma in sellar region	3	Unrelated	No	Recovered/resolved	Discontinued
	Asthenia	3	Unrelated	No	Not recovered/not resolved	None

* Patient discontinued after 6 doses and was therefore nonevaluable.

No hemorrhagic events were observed. The lowest PLT counts across all patients ranged from 29-67x10^9/L; no patient discontinued because of thrombocytopenia. No dose-limiting toxicities were observed. Reductions in palpable spleen length from baseline to the most recent postbaseline assessment were reported for 5/6 patients. **Conclusions.** In this phase 1b study of ruxolitinib in thrombocytopenic patients with MF, no DLT has occurred at the first 2 dose levels in patients with PLTs 75-100x10^9/L; thus, additional patients are being recruited for both strata. Updated results will be reported.

0370

CYTOREDUCTIVE TREATMENT PATTERNS FOR ESSENTIAL THROMBOCYTHEMIA IN EUROPE. ANALYSIS OF 3643 PATIENTS IN THE EXELS STUDY

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Background. The Evaluation of Xagrid Efficacy and Long-term Safety (EXELS) study is an observational cohort study of essential thrombocythemia (ET) in high-risk patients. It is being conducted in 13 European countries: Denmark, Finland, France, Germany, Greece, Ireland, Italy, Netherlands, Norway, Portugal, Spain, Sweden, and UK. The study is designed to monitor the safety and efficacy of cytoreductive therapies in routine clinical practice. The study is sponsored by Shire Pharmaceuticals. **Aims**. The objective of this analysis was to observe the disease characteristics and cytoreductive treatment patterns of ET across Europe. **Methods.** High-risk patients (>60 years of age; history of thrombosis; platelet count >1000x10^9/l) with ET who were receiving cytoreductive therapy at the time of registration were enrolled, following informed consent. The choice of cytoreductive therapy was determined prior to study registration and patients were managed according to local practice. Data were collected from each patient at the time of registration and every 6 months thereafter for 5 years, using an electronic data capture system. Here we describe findings from a data-cut taken in September 2011, scheduled 2.5 years since the last patient was enrolled. Approximately 70% of the patients were continuing in the study at this data-cut. **Results**. A total of 3643 patients, 61.3% females and 38.7% males, across a wide range of ages (<40 years, 6.9%; 40-59 years, 25.5%; ≥60 years, 67.6%) were enrolled into the study. The majority of patients (80.6%) had been previously treated with a cytoreductive therapy at the time of registration. Time from diagnosis to study registration was 0-<2 years for 32.9% of patients; 2-<5 years for 26.8%; 5-<10 years for 24.5%; and ≥10 years for 15.8% of patients. Hydroxycarbamide and anagrelide were the two main cytoreductive treatments prescribed as monotherapy in 65.1% and 22.2% of patients, respectively. 'Other' treatments included interferon, busulphan, pipobroman, 32P, and hydroxycarbamide and anagrelide in combination. At registration, a greater proportion of patients in the anagrelide group were <60 years (59.3%), relative to those taking hydroxycarbamide (19.3%). The full distribution of patient age according to treatment type were as follows: hydroxycarbamide (<40=2.4%; 40-59=16.9%; ≥60=80.7%); anagrelide (<40=16.3%; 40-59=43.0%; ≥60=40.8%); others (<40=12.9%; 40-59=39.4%; ≥60=47.7%). The number of patients and type of treatment at registration, per country, is displayed in Figure 1.

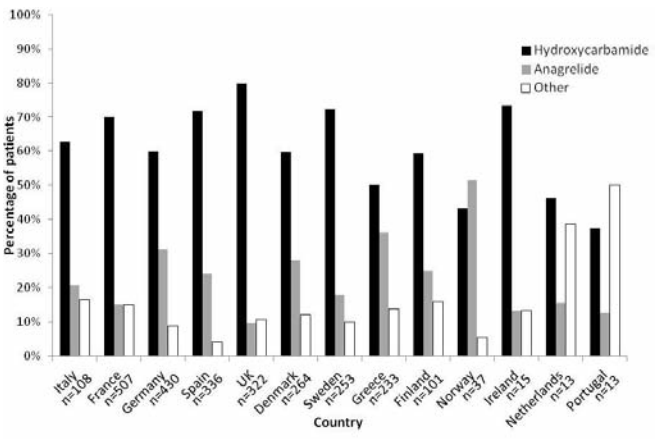

Figure 1. Type of treatment at registration per country.

The treatment pattern between countries was notably different, with rates of use showing marked variation; hydroxycarbamide 38-80%, anagrelide 10-51%, other 4-50%. **Summary and Conclusions.** This study provides real-world evidence regarding the cytoreductive treatment patterns of ET across Europe, taken from the largest observational cohort of high-risk patients with ET. Hydroxycarbamide was the most frequent treatment of choice in nearly all of the participating countries across Europe, but patient age strongly influenced the choice between hydroxycarbamide and anagrelide therapy. In general, the treatment pattern is in accordance with expert recommendations in Europe. The study is due to be completed in June 2014, and is expected to provide valuable information on long-term safety and efficacy of different cytoreductive treatments, with regard to disease-related events and toxicity.

0371

ADVERSE EVENTS AND THE RETURN OF MYELOFIBROSIS-RELATED SYMPTOMS AFTER TREATMENT INTERRUPTION OR DISCONTINUATION IN THE COMFORT-I STUDY

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Background. Ruxolitinib is a JAK1/JAK2 inhibitor that has demonstrated significant clinical benefit in patients with myelofibrosis in the COMFORT-I study. **Aims.** To assess adverse events after interruption or discontinuation of study therapy and to evaluate the return of myelofibrosis-related symptoms after interruption of ruxolitinib therapy in the COMFORT-I study. **Methods.** COMFORT-I (NCT00952289) is a phase III, randomized (1:1), double-blind, placebo-controlled study in 309 adults patients with International Prognostic Scoring System intermediate-2 or high-risk primary myelofibrosis, post-essential thrombocythemia myelofibrosis, or post-polycythemia vera myelofibrosis. Patients assessed myelofibrosis-related symptoms daily using the modified Myelofibrosis Symptom Assessment Form v2.0; Total Symptom Score (TSS) was calculated from individual scores for abdominal discomfort, pain under left ribs, early satiety, night sweats, itching, and bone/muscle pain. Therapy was interrupted if platelet or absolute neutrophil count fell below 50,000/μL or 500/μL, respectively. Adverse events were evaluated after treatment interruption and treatment discontinuation. The study protocol suggested an optional tapering strategy and possible addition of steroids if ruxolitinib therapy was discontinued for reasons other than thrombocytopenia. All patients provided written informed consent. **Results.** The mean duration of treatment interruption was 16 days in the ruxolitinib group and 9 days in the placebo group. In ruxolitinib-treated patients with treatment interruption, TSS gradually returned to baseline levels over approximately 1 week. The most common adverse event leading to treatment interruption or discontinuation in each group was thrombocytopenia (n=11, ruxolitinib) and abdominal pain (n=4, placebo). Of these, only 1 ruxolitinib-treated patient discontinued therapy for thrombocytopenia. A summary of new onset/worsening of adverse events after treatment interruption or discontinuation is presented (Table 1). Of 103 patients who interrupted study treatment, 6 patients (n=3, ruxolitinib; n=3, placebo) experienced a total of 8 serious adverse events (4, ruxolitinib; 4, placebo). Of 58 patients who discontinued study treatment, 23 patients (n=10, ruxolitinib; n=13, placebo) experienced a total of 43 serious adverse events (19, ruxolitinib; 24, placebo). Analysis of serious adverse events of each patient showed that there was no specific pattern or difference between treatment groups after treatment interruption or discontinuation. Four of 21 ruxolitinib-treated patients had dose tapering following study drug discontinuation.**Summary/ Conclusions.** Apart from the expected return of myelofibrosis-related symptoms, there was no pattern of adverse events to suggest that interruption or discontinuation of ruxolitinib therapy is associated with a specific withdrawal syndrome. *Supported by Incyte Corporation.* Dr. Deininger is currently at the Division of Hematology and Hematologic Malignancies and Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

Table 1. Summary of adverse events (AEs) and serious adverse events (SAEs) after treatment interruption or discontinuation in COMFORT-I

	Ruxolitinib (N=155)	Placebo (N=151)
Treatment interruption, n	49	54
Grade ≥3 AEs, n	8	7
SAEs, n	3	3
	Gastrointestinal hemorrhage; Fatigue, neutropenic fever; Urosepsis	Anemia; Pulmonary edema; Hepatic encephalopathy, acute gout
Treatment discontinuation, n	21	37
Grade ≥3 AEs, n	12	17
SAEs, n	10	13
	Fall, splenic hemorrhage; Subdural hematoma; Pyrexia; Pneumonia, respiratory failure, septic shock; Acute myeloid leukemia (AML); Thrombocytopenia, renal failure; Pneumonia, Clostridial infection, sepsis; Pneumonia; Fatigue, muscular weakness, splenic infarction; Thrombocytopenia, AML	Staphylococcal infection; Gastrointestinal hemorrhage; Abdominal pain; Colitis, pulmonary embolism; Urinary tract infection, pneumonia; Dehydration, fall, splenic hematoma, subdural hematoma, cardiac failure, leukocytosis, pneumonia, renal failure, sepsis; Abdominal pain; Myelofibrosis; Renal failure; Pulmonary edema, multiorgan failure; Disease progression; Disease progression; Intestinal ischemia

NOTE: AEs and SAEs reported are as of the data cutoff for the primary endpoint of the study.

0372

COMPARISON OF THE EFFICACY OF PLACEBO AND BEST AVAILABLE THERAPY FOR THE TREATMENT OF MYELOFIBROSIS IN THE COMFORT STUDIES

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Background. Ruxolitinib is a potent and selective oral JAK1/2 inhibitor that has been approved in the US and has demonstrated rapid and durable reductions in splenomegaly and improved disease-related symptoms and quality of life (QoL) in 2 phase 3 studies (COMFORT-I and -II) in patients with primary MF, post-polycythemia vera-MF, or post-essential thrombocythemia-MF. Compared with the control group in each study (COMFORT-I, placebo; COMFORT-II, best available therapy [BAT]), a significantly higher proportion of patients receiving ruxolitinib achieved a ≥35% reduction in spleen volume at week 24 (COMFORT-I and -II; *P*<.0001) and week 48 (COMFORT-II; *P*<.0001). **Aims.** This analysis compares the efficacy outcomes between the placebo arm from COMFORT-I and the BAT arm from COMFORT-II. **Methods.** COMFORT-I is a randomized (1:1), double-blind, multicenter study comparing ruxolitinib 15 or 20mg twice daily (BID) with placebo, and COMFORT-II is a randomized (2:1), open-label, multicenter study comparing ruxolitinib 15 or 20mg BID with BAT (investigator-selected therapy, including no treatment). QoL was measured using patient responses with the EORTC QLQ-C30 as an exploratory endpoint in both studies. **Results.** In the COMFORT-I and COMFORT-II studies, 154 patients received placebo (ruxolitinib, n=155), and 73 patients received BAT (ruxolitinib, n=146), respectively, and were included in the primary efficacy analyses. The demographic and baseline characteristics were similar between the control arms of the 2 studies including spleen size below the costal margin (mean [standard deviation], 16.4 [6.27] cm and 15.8 [6.71] cm in placebo and BAT, respectively). Only 1 patient (0.7%) who received placebo and no patients who received BAT had a ≥35% reduction in spleen volume from baseline at week 24 (Figure). The median percent increases in spleen volume from baseline to week 24 in both the placebo and BAT groups were numerically similar (placebo, 8.5% [range, -46.4% to 48.8%]; BAT, 5.1% [range, -33.3% to 29.7%]). In contrast, almost all patients who received ruxolitinib

had a reduction in spleen volume from baseline at week 24 (COMFORT-I: median, -33.0%; COMFORT-II: median, -27.5%).The QLQ-C30 provides a measurement of QoL and MF-related symptoms, including fatigue, pain, dyspnea, insomnia, and appetite loss. At 24 weeks, neither the placebo nor BAT arms had clinically meaningful changes from baseline (10 points [Osoba et al. *J Clin Oncol*. 1998]) in global QoL (decreases indicate worsening) or symptom scales (increases indicate worsening) (Table). Although differences exist between the placebo and BAT arms in the mean change from baseline, between-group comparison was not performed because of the large standard deviations that complicate any comparisons between the 2 groups. **Conclusions.** This post hoc analysis shows that patients who received BAT in the COMFORT-II study fared no better in clinically meaningful QoL responses and had numerically similar increases in spleen size as those who received placebo in COMFORT-I. No clinically meaningful improvements in QoL or symptoms were seen on either the placebo or BAT arms. These new data strongly suggest that traditional therapies for MF provide little improvement in spleen size, symptoms, or QoL as compared with placebo.

Table. Mean EORTC QLQ-C30 Global Health Status and Subscale Results at Week 24 i the Placebo and BAT Arms (Observed Cases)[a]

	Placebo		BAT	
	n	Mean change from baseline at week 24 (SD)	n	Mean change from baseline at week 24 (SD)
Global health status/QoL	104	-3.4 (21.53)	37	5.2 (23.76)
Fatigue	107	1.8 (24.71)	39	-1 (23.57)
Pain	104	8.3 (27.47)	39	3 (28.06)
Dyspnea	105	1 (27.53)	38	5.3 (31.51)
Insomnia	105	-2.2 (32.12)	39	6 (28.48)
Appetite loss	107	0.6 (33.96)	39	0.9 (34.61)

[a]For patients with measurements at both baseline and week 24.
BAT, best available therapy; EORTC QLQ-C30, European Organization for the Research an Treatment of Cancer Quality of Life Questionnaire.

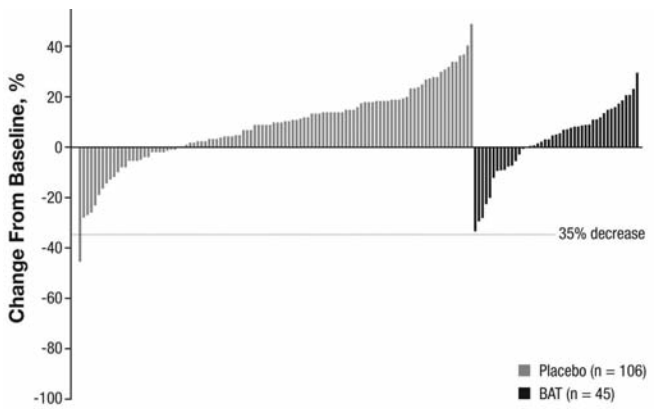

Figure. Percent Change From Baseline in Spleen Volume at Week 24 for Individual Patients

0373

REDUCTIONS IN JAK2V617F ALLELE BURDEN WITH RUXOLITINIB TREATMENT IN COMFORT-II, A PHASE III STUDY COMPARING THE SAFETY AND EFFICACY OF RUXOLITINIB TO BEST AVAILABLE THERAPY (BAT)œ

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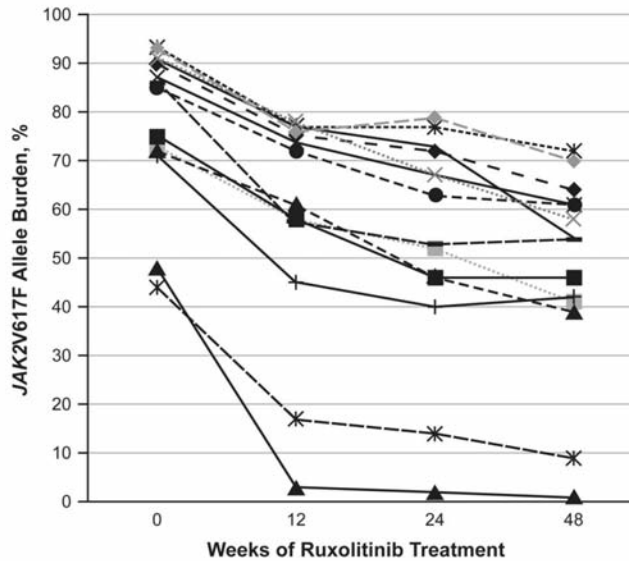
Background. Ruxolitinib is a potent and selective JAK1/2 inhibitor approved in the United States based on results of the phase 3 COMFORT studies. Ruxolitinib demonstrated rapid and durable reductions in splenomegaly and improved MF-related symptoms and quality of life of patients with MF. **Aims.** To determine the correlation between changes in mutant allele burden (%V617F) with spleen size reduction in COMFORT-II. **Methods.** COMFORT-II is a randomized, open-label, phase III study comparing ruxolitinib 15 or 20 mg twice daily (BID) with BAT. The primary endpoint was a ≥ 35% reduction in spleen volume from baseline at week 48. Change in %V617F was measured by allele-specific qPCR. Patients were stratified by absolute reduction in %V617F (< 10%, 10% to 20%, > 20%) and results were correlated with achievement of the primary endpoint. **Results.** More patients in the ruxolitinib arm had ≥ 10% V617F reductions compared with BAT (41% vs 5%; *P* = .01; Table). The majority of reductions > 20% were gradual and progressive over the course of the study; 2 patients had rapid reductions from 48% to 1% and 45% to 9% over 48 weeks (Figure). In the ruxolitinib arm, significantly more patients with a > 20% V617F reduction achieved the primary endpoint compared with patients with a < 10% reduction (79% vs 30%; *P* = .004). For patients with < 10% reductions (15 mg BID, n = 16; 20 mg BID, n = 24), the average total daily dose (TDD) of ruxolitinib was 29.6 mg; patients with > 20% reductions (15 mg BID, n = 3; 20 mg BID, n = 11) had a TDD of 35.3 mg. **Conclusions.** Patients who received ruxolitinib had larger reductions in *JAK2*V617F allele burden compared with BAT. %V617F reductions were gradual over the course of the 48-week study; longer follow-up is needed to determine the extent of allele burden reduction.

Table. Reductions in *JAK2*V617F Allele Burden at Week 24 and Week 48.

n (%)		Ruxolitinib (n = 145)[a]	BAT (n = 69)[a]
*JAK2*V617F positive at baseline		110 (76) Mean: 73.5 Median: 84.5 Range: 5-96	49 (71)[b] Mean: 69.1 Median: 81 Range: 1-95
*JAK2*V617F positive with week 24 data available		75 (68)	24 (49)
%V617F reduction	Median	-6.0%	0.5%
	< 10%[c]	46 (61)	24 (100)
	10% to 20%	15 (20)	0
	≥ 20%	14 (19)	0
*JAK2*V617F positive with week 48 data available		68 (62)	22 (45)
%V617F reduction	Median	-7.0%	0%
	< 10%[d]	40 (59)	21 (95)
	10% to 20%	14 (21)	1 (5)
	≥ 20%	14 (21)	0

[a] Patients with %V617F data.
[b] Unknown JAK-mutation status, n = 4.
[c] Includes patients with increased %V617F (range, 0%-11%).
[d] Includes patients with increased %V617F (range, 0%-7%).



[a]Each line represents a single patient.

Figure. Absolute *JAK2*V617F Allele Burden[a]

0374

THROMBOTIC MANIFESTATIONS IN 1161 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV) DIAGNOSED BETWEEN 16 AND 40 YEARS OF AGE: A RETROSPECTIVE STUDY OF THE GIMEMA MPN-WG

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Background. Philadelphia negative myeloproliferative neoplasms (MPN) are rare in patients younger than 40 years, and information on the natural history of disease is lacking in this setting. In particular, the available evidence suggests that these patients might have distinct vascular complications whose proper understanding is crucial for clinical decision making.The *aim* of this study, performed under the auspices of the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA), was to evaluate thrombotic events and their risk factors in a large cohort of patients with MPN diagnosed between 16 and 40 years of age. **Material and Methods.** Nine Italian hematological centers completed an ad-hoc supplied electronic sheet, reporting the clinical and laboratory data of their young patients with MPN (age range 16 and 40 years). The final cohort included 1161 patients (61% females, median age at diagnosis 32.8 years) of whom 929 with ET and 232 with PV diagnosed between 1960 and 2011. Diagnostic criteria used were those in effect at the time of initial diagnosis; the Polycythemia Vera Study Group (PVSG) criteria were preferentially used for diagnoses prior to 2006 (570 patients), while the WHO criteria were for diagnoses thereafter (592 patients). We applied X^2 test for categorical variables and Mann-Whitney U for continuous variables. To evaluate risk factors for thrombosis we applied logistic regression analysis.

Table 1.

	Unusual site N=76	DVT or PE N=31	Univariate p
General characteristics			
Sex F (%)	52 (68%)	19 (61%)	0.67
Median age at diagnosis	30.4	34	0.114
Median follow up (years)	10.6	11.7	0.382
WBC x 10^9/L	9.9±4.2	10.6±5.5	0.856
Hb g/L	14.7±2.7	15.3±2.5	0.542
HT (%)	43.8±8.8	46.7±6.5	0.338
Platelets x 10^9/L	649±389	727±302	0.123
JAK2V617F	51/57 (89%)	13/23 (56%)	0.005

Results. In a total of 203 patients (17.4%) arterial (n=96, 8%) or venous (n=107, 9%) thrombosis was recorded, with higher prevalence in those with PV (27 % vs 15%, p<0.05). Considering venous thrombosis, 76 were thrombosis at unusual site (67 splanchnic vein thrombosis and 9 cerebral vein thrombosis) and 31 were deep vein thrombosis (DVT) or pulmonary embolism (PE), as reported in the table. Venous thrombosis was the presenting feature of MPN in 41 (38%) patients and thrombosis at unusual site was more common than DVT as presenting feature of MPN (p=0.009). Multivariable analysis identified diagnosis of PV (RR 2.9, 95% CI 1.2-6.9) and presence of *JAK2* (V617) (RR 5.5, 95% CI 1.6-18.9) as a significant risk factors for thrombosis at unusual site, and this was true also for female gender within ET patients (RR 7.8, 95% CI 1.02-60.2). The only predictor of DVT or PE seemed to be leukocytosis (>11x10^9/L; 3.0, 95% CI 0.96-9.4) without reaching statistical significance. Among arterial events, TIA or stroke occurred in 5% of ET and 6 of PV and acute coronary syndrome in 2% and in 7% respectively with the latter difference statistically significant (p<0.001). The presence of cardiovascular risk factors was the only predictor for arterial thrombosis in ET patients. **Discussion.** The most common phenotypic manifestation of venous thrombosis in young patients with MPN is thrombosis at unusual site, particularly in ET females, thus suggesting a possible interaction with hormonal risk factors. Most of these cases of atypical vein thrombosis occur in patients carrying the *JAK2* (V617F) mutation. Overall, PV has a more aggressive clinical course, characterized by an increased prevalence of thrombosis compared to ET.

0375

PROGNOSTIC SIGNIFICANCE OF GENETIC ABERRATIONS IN SECONDARY ACUTE MYELOID LEUKEMIA

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Background. Chronic myeloproliferative neoplasms and myelodysplastic syndromes can evolve to secondary acute myeloid leukemia (sAML). It has been shown that sAML can arise either on the background of the chronic phase founder clone, or as an independent event, resembling *de novo* AML (dnAML). The leukemogenesis in sAML is poorly understood and these patients have poor prognosis. **Aims.** The main aim of the study was to compare genetic lesions in sAML and dnAML patients, and identify those with the most prominent impact on the overall survival (OS). Furthermore, we aimed to analyze the interplay of genetic aberrations in different genomic regions and their potential use in patient stratification. **Methods**. High-resolution genotyping and loss of heterozygosity mapping was performed on DNA samples from 86 sAML and 117 dnAML patients, using Affymetrix SNP 6.0 arrays. TP53, RUNX1, CBL, IDH1/2, NRAS, NPM1 and FLT3 genes were analyzed individually for mutations in all patients. OS comparisons were performed using the log-rank test, and multivariate survival analysis using Cox's regression model. Mutual information and interaction information analysis were performed using the R package "infotheo". **Results.** Patients with sAML showed higher karyotype complexity than dnAML (P<0.001), and more rarely a normal karyotype (P=0.002). We identified a total of 669 deletions, gains and acquired uniparental disomies (UPD). There were 36 recurrent aberrations (>5 events), most of which associated with sAML. High number of events allowed fine mapping (<1Mb) of target genes, such as JARID2, IKZF1, NF1. Mutations in TP53, 9pUPD, and del7q (harboring CUX1) were significantly associated with sAML, while NPM1 and FLT3 mutations were significantly associated with dnAML. Mutations in IDH1/2 were mutually exclusive with TP53 and NRAS mutations.Patients with sAML had worse 3-year OS rate (11.3%±4.9%) compared to dnAML (35.2%±5.3%; P=0.003). We analyzed the influence of features not associated with chronic phase or dnAML on OS of sAML patients. SAML patients with TP53 mutations showed a significantly shorter OS (1.8 months, 95%CI, 0.8-2.8) compared to patients with wtTP53 (5.6 months, 95%CI, 1.5-9.7). Patients carrying TP53 mutations demonstrated a lower 1-year OS rate then those with wtTP53 (7.1%±6.9% vs. 32.9%±7.1%; P=0.002). Complex karyotype, del7q (CUX1) and del7p (IKZF1) showed no significant effect on survival of sAML patients. Multivariate analysis confirmed that mutation in TP53 was the only independent adverse prognostic factor for OS (hazard ratio 2.41; 95%CI, 1.20-4.82; P=0.013) in sAML.The mutual information analysis showed that del5q and TP53 mutations, del12p (ETV6) and del5q, 11q gains and del17q, associate with respect to diagnosis. The negative interaction information analysis, indicating exclusivity to sAML, showed association for del5q and 9pUPD, del5q and TP53 mutations, as well as del20q and del4q (TET2). **Conclusions.** Our data suggests that distinct genetic lesions drive leukemogenesis in sAML, compared to dnAML. High karyotype complexity of sAML patients does not influence their OS, and indicates the presence of mainly passenger aberrations. Somatic mutations in TP53 are the only independent adverse prognostic factor in sAML. The mutual information and interaction analysis identified several potential markers for genetic stratification of patients with AML.

0376

THE PROGRESSIVE BURDEN OF MYELOFIBROSIS (MF) IN UNTREATED PATIENTS: AN ASSESSMENT OF PATIENT-REPORTED OUTCOMES IN PLACEBO-TREATED PATIENTS FROM COMFORT-I

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Background. COMFORT-I is a Phase III randomized, double-blind, placebo-controlled study of ruxolitinib in patients with MF, which incorporated patient-reported outcomes (PROs) and other quality of life measures to assess MF symptom burden. The placebo arm provides a controlled setting to assess changes in symptom burden among patients not receiving MF therapy. **Aims.** To assess the changes in PROs among patients from the COMFORT-I study not receiving treatment for MF. **Methods.** Informed written consent was obtained from all enrolled patients. Adult patients with intermediate-2 or high-risk MF (primary MF, post-polycythemia vera MF, and post-essential thrombocythemia MF) by International Prognostic Scoring System classification with ≥6 months life expectancy were randomized to placebo or ruxolitinib (1:1) after a 28-day washout of previous MF therapies. Individual MF symptom severity (night sweats, itching, abdominal discomfort, pain under ribs on left side, early satiety, and bone/muscle pain) were reported by the patient using the modified Myelofibrosis Symptom Assessment Form v2.0 electronic diary, and a Total Symptom Score (TSS) was calculated based on the sum of daily individual symptom scores. Other PRO measures included the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30 [100-point normalized scale; lower scores indicate poorer quality of life]); Patient Reported Outcome Measurement Information System (PROMIS) Fatigue Scale (100-point normalized scale; higher scores indicate greater fatigue); and Patient Global Impression of Change (PGIC; 7-point scale: 1=very much improved, 4=no change, 7=very much worse). Spleen volume was measured using MRI or CT and evaluated by a central blinded reviewer at baseline and every 12 weeks. Patients in the placebo group who had a ≥25% increase from baseline in spleen volume accompanied by worsening MF symptoms (early satiety with weight loss or worsening spleen pain requiring daily narcotic use) before database lock were eligible for unblinding and cross over to ruxolitinib. Patients who withdrew or crossed over to ruxolitinib before a study visit were not included in analyses for that visit or subsequent visits. **Results.** The COMFORT-I placebo arm included 154 patients with a median of 2.5 years since diagnosis; 35% were intermediate-2 and 65% high-risk. By week 24, mean TSS worsened by 42% from baseline. EORTC QLQ-C30 Global Health Status and functional subscale scores also worsened from baseline to week 24, with the greatest changes in Role Functioning (mean change = -11.1) and Social Functioning (mean change = -9.0); mean PROMIS Fatigue score worsened by 9.1%. Mean PGIC score was 4.2 at week 24; 40.2% of patients perceived their condition to be worse and 29.9% unchanged. The majority of patients experienced an increase in spleen volume; median increases at weeks 12 (n=132), 24 (n=106), and 36 (n=46) were 6.1%, 8.5%, and 9.7%, respectively. **Conclusions.** Patients receiving placebo reported worsening of MF symptoms and other PROs. Increases in spleen volume over time were also observed. These findings illustrate the progressive and debilitating nature of MF and suggest that early intervention with effective therapy should be considered. *Supported by Incyte Corporation.*

0377

TELOMERE LENGTHS IN PATIENTS WITH JAK2V617F: AN AGE-ADJUSTED MODEL

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Background. Myeloproliferative neoplasms (MPNs) exhibit the overproduction of peripheral blood cells caused by clonal disorders in the hematopoietic cells. An acquired mutation, *JAK2*V617F, has become the most common molecular abnormality in 95-97% of polycythemia vera (PV), 50-60% of essential thrombocytosis, and 50% of primary myelofibrosis. Telomeres are thousands of repeated sequences (TTAGGG)n capped at the end of chromosomes, essential for protecting chromosome from the fusion events. Recently, the reduction of telomere length has been found in the MPN patients with *JAK2*V617F. However, the individual telomere length might be modulated by several factors, such as the age, the cell type. These factors might challenge the accuracy of the comparisons between the MPN patients and the healthy controls. **Aims.** To generate a formula for the telomere length measurement based on age and to assess the variation of telomere length in patients with *JAK2*V617F.Method: A total of 255 normal controls and 63 patients diagnosed with MPN were enrolled in this study. This study was approved by the Institutional Review Board of the NTUH, and written informed consent was obtained from all the participants. Quantitative-PCR accompanying with ratio of telomere product (T) to single-copy gene product (S) were used. **Results.** Among 255 normal individuals, 219 were males and 36 were females and with the age of 9 to 81. The T/S ratios of males were lower than those of females (0.76 ± 0.38 vs. 0.94 ± 0.41, p=0.0037). There were no significant difference of T/S ratios between paired DNA from granulocytes and mononuclear cells (0.97 ± 0.37 vs. 0.83 ± 0.27, p=0.3601). When divided into 4 groups based on age (<27 y/o, 27-45 y/o, 46-64 y/o, >64 y/o), there were significant difference among these four groups by using one-way ANOVA test (p<0.0001). Therefore, the relationship between the age and the telomere length need a scientific model. The R-program implemented in the S-plus software (TIBCO Software Inc) was applied to fit the regression curve between ages and telomere lengths for the 255 normal individuals and the polynomial with third degree did have the best significance rather than the polynomial with first and second degrees (Figure 1). The formula of this polynomial was $y=9.06*10^{-5}x^3-0.0011x^2+0.0251x+1.13$. The p-value of F-statistic which tested the significance of this regression curve was 0.0015 and the proportion which could be explained by the fitted model was 0.363. Of 63 patients with MPN, 35 patients (55.6%) were positive of *JAK2*V617F. The telomere length of each MPN patient with *JAK2*V617F was compared with the corresponding age-matched T/S generated by the formula of the regression line, demonstrating a significant difference from the normal control cohort (0.42 ± 0.18 vs. 0.72 ± 0.20, p=0.0363. sensitivity = 0.77). Furthermore, the T/S ratios displayed a significant difference between paired DNAs from granulocyte and mononuclear cell in *JAK2*V617F patients (0.52 ± 0.44 vs. 0.89 ± 0.45, p=0.0287). **Conclusions.** We concluded that adjusted with age, the telomere lengths of patients with *JAK2*V617F were shorter than those of normal controls and displayed a significant difference between granulocytes and mononuclear cells.

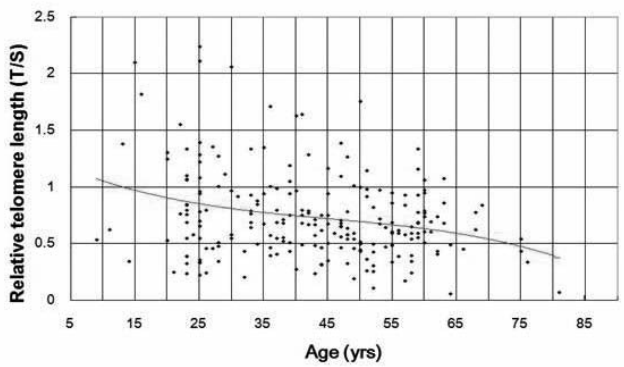

Figure 1. The spots displayed a wide variation in the same age, and predicted polynomial of third degree was showed on the scatter plot.

0378

HEALTH-RELATED QUALITY OF LIFE (HRQOL) AND SYMPTOM BURDEN IN PATIENTS WITH MYELOFIBROSIS (MF) IN THE COMFORT-II STUDY

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Background. Ruxolitinib has demonstrated rapid and durable reductions in splenomegaly and improved disease-related symptoms and QoL in 2 phase 3

studies (COMFORT-I and -II) in patients with primary MF (PMF), post-polycythemia vera-MF (PPV-MF), or post-essential thrombocythemia-MF (PET-MF) and has been approved in the US. The prevalence of individual symptoms among these patients has not been defined. **Aims.** This analysis evaluated baseline HRQoL and symptoms among patients enrolled in COMFORT-II. **Methods.** COMFORT-II is a randomized, open-label, multicenter, phase 3 study comparing ruxolitinib with best available therapy. HRQoL and symptoms were assessed at baseline using the European Organisation for the Research and Treatment of Cancer QoL Questionnaire-Core 30 (EORTC QLQ-C30) and Functional Assessment of Cancer Therapy-Lymphoma (FACT-Lym); this analysis summarizes these scores for all patients, regardless of assigned treatment. **Results.** In COMFORT-II (N = 219), 52% of patients were aged > 65 years and 57% were male. By International Prognostic Scoring System criteria (Cervantes et al. *Blood.* 2009), 40% had intermediate-2-risk MF, and 60% had high-risk MF.Patients had a mean palpable spleen length of 15.2 cm (range, 5-37 cm) below the costal margin. Patients with PMF or PPV-MF had larger spleens (mean, 15.8 cm; 95% CI, 14.6-16.9 cm and 16.2 cm; 95% CI, 14.5-17.9 cm, respectively) than those with PET-MF (11.4 cm; 95% CI, 9.7-13.1 cm).The most frequent symptoms (reported as "quite a bit"or "very much") were fatigue (54%), dyspnea (30%), insomnia (30%), pain (29%), night sweats (23%), and itching (21%), and there were differences in baseline symptoms across MF subtypes (Table, upper panel). Mean EORTC QLQ-C30 functioning, global health status/QoL, and symptom scores were comparable to or worse than those that have been reported for patients with acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and breast cancer (Table, lower panel). **Conclusions.** This analysis shows that patients with MF experience severe disease-related symptoms and have diminished HRQoL similar to those with AML, but because patients with MF have a longer life expectancy (an average of 2.3 to 4 years for high- and intermediate-2-risk patients, respectively), they may suffer with a reduced QoL for many years.

Table. Prevalence of Symptoms at Baseline in COMFORT-II by MF Subtype (upper panel); Mean EORTC QLQ-C30 Scores at Baseline in COMFORT-II and for Patients With AML, CML, and Breast Cancer (lower panel).

% "quite a bit" or "very much"	MF (N = 219)	PMF (n = 116)	PPV-MF (n = 68)	PET-MF (n = 35)
Fatigue	54.2	59.2	46.1	53.2
Dyspnea	29.5	28.7	30.2	31.2
Insomnia	29.7	32.7	25.4	28.2
Pain	29.2	25.9	28.6	41.9
Night sweats	22.7	21.3	27.3	18.2
Itching	21.4	16.8	28.8	21.3
Weight loss	14.6	12.1	22.7	6.3

Mean (SD) EORTC QLQ-C30 score	MF (N = 219)	AML (N = 155)[a]	CML (N = 73)[b]	Breast Cancer (N = 225)[c]
Functional scales[d]				
Physical functioning	68.0 (22.9)	--	78.0 (21.0)	86 (17)
Role functioning	66.2 (31.0)	--	78.1 (36.3)	85 (27)
Cognitive functioning	78.6 (23.5)	82.2 (18.9)	86.1 (22.1)	83 (20)
Emotional functioning	73.6 (23.6)	86.1 (18.5)	78.8 (22.6)	67 (21)
Social functioning	78.1 (27.0)	66.1 (31.0)	84.3 (25.7)	84 (21)
Symptom scales[e]				
Fatigue	46.6 (28.1)	36.2 (22.7)	29.8 (25.4)	28 (21)
Pain	25.9 (29.9)	13.7 (20.4)	10.1 (19.6)	23 (22)
Nausea/vomiting	5.9 (14.3)	9.0 (18.3)	5.0 (12.9)	6 (12)
Dyspnea	35.8 (32.8)	11.3 (17.1)	15.5 (20.9)	15 (21)
Insomnia	33.7 (34.1)	20.4 (26.1)	26.9 (32.7)	27 (27)
Appetite loss	18.2 (28.2)	18.0 (30.5)	13.7 (28.8)	13 (22)
Constipation	9.3 (20.4)	7.9 (19.1)	9.6 (19.6)	11 (22)
Diarrhea	16.8 (26.6)	12.6 (25.1)	7.3 (19.4)	6 (14)
Global health status/QoL[d]	53.7 (21.8)	--	70.2 (21.5)	45 (30)

[a] Scott NW, et al. *EORTC QLQ-C30 Reference Values*. 2008.
[b] Homewood J, et al. *Hematol J*. 2003;4(4):253-262.
[c] Martinelli F, et al. *Expert Rev Pharmacoecon Outcomes Res*. 2011;11(5):587-599.
[d] Higher scores indicate better functioning and QoL.
[e] Higher scores indicate more severe symptoms.

0379

ASSOCIATION OF CYTOKINE LEVELS AND REDUCTIONS IN SPLEEN SIZE IN COMFORT-II, A PHASE 3 STUDY COMPARING RUXOLITINIB TO BEST AVAILABLE THERAPY (BAT)

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Background. Ruxolitinib is a potent and selective oral JAK1/2 inhibitor that has been approved in the United States. Ruxolitinib has demonstrated rapid and durable reductions in splenomegaly and improved disease-related symptoms and quality of life (QoL) in two phase 3 studies (COMFORT-I and -II) in patients with primary MF (PMF), post-polycythemia vera-MF (PPV-MF), or post-essential thrombocythemia-MF (PET-MF). **Aims.** This analysis evaluated associations between cytokine levels and spleen size reductions in the COMFORT-II study. **Methods.** COMFORT-II is a randomized (2:1), open-label, phase 3 study comparing the safety and efficacy of ruxolitinib with BAT. Spleen volume was measured by MRI every 12 weeks and spleen length by palpation at each study visit. Plasma samples were analyzed using Rules-Based Medicine's Human MAP®v1.6; 89 cytokines were measured at baseline and weeks 4, 24, and 48. Simple linear regression was used to evaluate the correlation between baseline or change from baseline in cytokine levels with percent change of spleen size and change in FACT-Lym symptom scores in a univariate manner. **Results.** In the ruxolitinib arm, association was found between changes in TNF-α and leptin levels and percent spleen volume reduction at weeks 24 and 48 that were not observed with BAT and were independent of *JAK2*V617F status. A decrease from baseline TNF-α was associated with greater spleen volume reduction at weeks 24 and 48 (Figure A). Higher baseline leptin levels were associated with greater percent spleen length reductions at week 48. Larger increases in leptin at week 4 were also associated with enhanced spleen response. Higher baseline leptin levels were associated with improvement in FACT-Lymphoma symptom self-assessment of weight loss at week 24 ($P < .01$) and week 48 ($P = .02$). A clear trend was observed for increased leptin levels at week 4 that preceded weight gain on ruxolitinib treatment (Figure B). **SUMMARY/ Conclusions.** This analysis has shown statistically significant associations between both changes in TNF-α and spleen size reduction, and leptin levels and spleen size reduction/weight gain.TNF-α levels have been shown to be elevated in patients with MF, independent of *JAK2*V617F status. TNF-α is known to suppress the growth of hematopoietic progenitors and to promote the growth of mutant clones, contributing to cytopenias associated with MF. The reduction of TNF-α by ruxolitinib has the potential to modify the disease through suppression of the mutant clone. (Fleischman et al, *Blood.* 2011; 118 (24): 6392-6398.)Patients with MF have abnormally low leptin levels due to disease-associated cachexia. The increase in leptin levels on study precedes weight gain. Further analyses are required to understand whether the leptin increase is an early marker of symptom resolution or a surrogate for JAK2 inhibition that may play a regulatory role in leptin production, rather than a causal effect. These observations require confirmation in an independent data set. In addition, a multivariate approach to fully explore the relationship between cytokines and clinical changes will be presented.

Figure A. Change in TNF-α levels vs spleen volume in ruxolitinib-treated patients

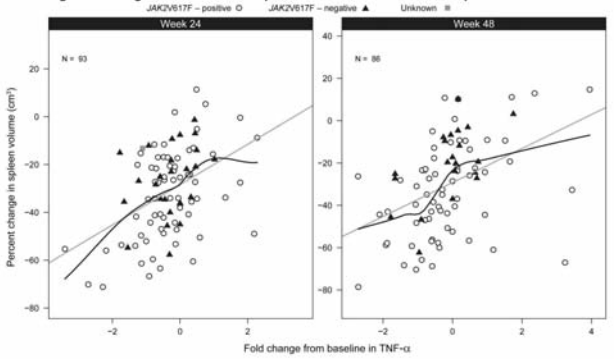
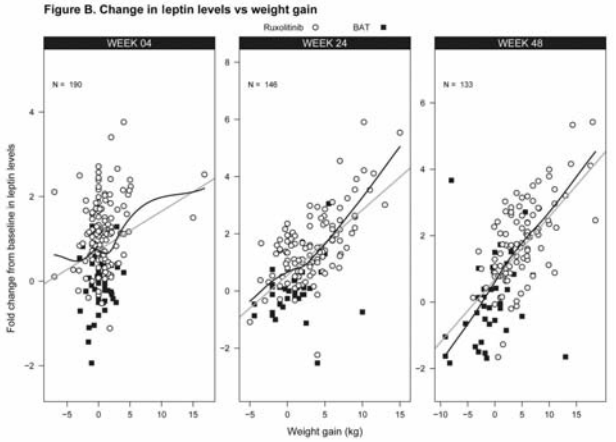


Figure B. Change in leptin levels vs weight gain



0380

MANAGEMENT OF MYELOFIBROSIS: A SURVEY OF CURRENT PRACTICE IN THE UNITED KINGDOM

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Background. Myelofibrosis is characterised by progressive anemia, pancytopenia, hepatosplenomegaly, fatigue, and night sweats. With the exception of stem cell transplantation (SCT), standard care focuses on palliation. There has been no previous evaluation of current management strategies in the UK. **Aims.** To assess current practices in management of Myelofibrosis in the UK.**Methods.** An internet based survey was designed to assess clinical need in Myelofibrosis, current management strategies and their success. This was emailed to over 800 Haematology clinicians and senior nurses within the UK. 105 healthcare professionals who had managed Myelofibrosis during the last 2 years responded.**Results.** Our survey confirmed that existing treatment options for Myelofibrosis are often unsatisfactory. When asked "In your experience, what are the three most common issues that need to be addressed during routine clinical reviews?", the majority (79%) of respondents replied that bothersome symptoms constituted the most common problem. Another 19% of respondents gave poor efficacy of medications as the most common issue. Moreover, Myelofibrosis particularly in advanced stages is a resource-intensive disease: patients with intermediate-risk 2 or high risk Myelofibrosis attended hospital every 3 to 4 weeks, according to 51.5% of respondents. Transfusion of blood products constituted the most common reason for hospital admission (43%) but sepsis (22.1%) and disease management (22.1%) were also significant.The most common complications of Myelofibrosis in over 50% of patients were anemia (68.1% of respondents), increased transfusion requirements (48.9%), and debilitating constitutional symptoms (30.9%). Splenic complications were also significant, however thrombosis and infection were uncommon.Current treatment tools for Myelofibrosis include splenectomy, SCT and drug therapies. Our study found that both splenectomy and SCT are rare treatment modalities. Regarding conventional drug therapies, our survey examined both their employment and their limitations. A wide variety of different therapies are utilised (Figure 1), suggesting there is a lack of treatment standardisation, and none of these treatments are uniformly successful. Indeed, only 32.1% of respondents stated therapy for anemia achieved its objective partially or completely in over 50% of patients. Moreover, the majority (78.8%) asserted that 50% or less of their patients maintained that response for over a year. A major finding was that many respondents thought there was no effective therapy for constitutional symptoms. For instance, a significant proportion of respondents considered there was currently no effective therapy for debilitating fatigue (40.7%), weight loss (34.6%), night sweats (25.9%) or fever (23.5%). **Conclusions.** This study constitutes an important source of information regarding current UK clinical practice. It identifies anemia, increased transfusion requirements and debilitating constitutional symptoms as the most frequent complications of Myelofibrosis. Two principal trends in management are prominent. Firstly, respondents used a wide variety of drugs, suggesting lack of standardisation and poor outcome. Secondly, effective management of symptom control is lacking. Further research is needed to clarify current treatment practices in the UK, and to assist the production of clinical guidelines.

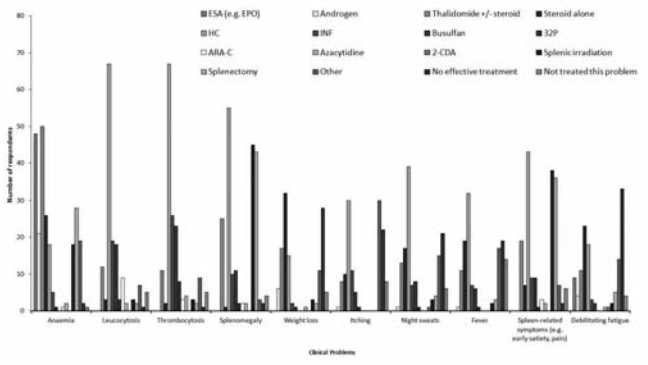

Figure 1.

0381

EFFECTIVENESS OF INTERFERON ALPHA THERAPY IN ESSENTIAL THROMBOCYTHEMIA

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Background. Interferon-alpha (IFN) has shown significant activity in MPN. In Essential Thrombocythemia (ET) this drug is able to rapidly normalize platelet and leukocyte counts, as well as organomegaly. IFN is the only therapy known to induce complete molecular remission with the reduction or the suppression of tumor burden.Despite its effectiveness, many patients discontinue IFN therapy due its side effects. Their incidence and severity are dose-related and the most common are flu like syndrome, myalgia, headache, fatigue, myelosuppression, liver abnormalities, autoimmune disease and depression. IFN has no leukemogenic effects and is safe in pregnancy.We hereby report our monocentric experience. **Methods.** From 1992 until today, the Division of Hematology of the Federico II University Medical School, Naples, Italy, followed 365 ET patients (diagnosis according to PVSG criteria and subsequently with 2008 WHO criteria); 257 patients were treated with cytoreduction: 64.5% with hydroxyurea, 23.5 % with IFN, 10% with anagrelide and 2% with busulfan.Patients treated with IFN received subcutaneous injection at induction dose of $3x10^6$ units 5 times a week, reduced on the basis of clinical-hematological response. **Results.** After a median follow up of 36 months, of the 60 patients receiving IFN, 60% achieved complete hematological response (CHR) according to the European Leukemia Net criteria; of these 13% were taken off therapy and 39% are still receiving very low doses of IFN ($3x10^6$ units every 7-15 days). As many as 20% experienced partial hematological response (PHR). The median time to response was 2.6 months.Response was not influenced by age (p=0.06), gender (p=0.4), baseline values of hematocrit and hemoglobin normalized for the gender, platelet count (p=0.29), WBC count (p=0.1). Furthermore $JAK2^{V617F}$ mutational status does not influence the response.Under IFN therapy no patients had thromboembolic or hemorrhagic event, independently of their cardiovascular risk. As many as 22 patients experienced flu-like symptoms, 3 dermatitis, 3 alopecia, 7 autoimmune thyroiditis; of them only 2 patients had hypothyroidism and had to suspend IFN treatment.Average spleen volume at the baseline was 495 ml; after a median follow-up of 18 months it was 472 ml, with no differences in volume enlargement in CHR/PHR compared to non responsive patients.Bone marrow fibrosis grade (according to Thiele) did not worsen after IFN treatment in either patients who achieved CHR or PHR. We are unable to evaluate data about non responsive patients, because this parameter could be influenced by their second line treatment. **Conclusions.** IFN was safe and effective for patients with ET. The ability of IFN therapy to control and modify the clinical course of the disease is confirmed by our data. This response is sustained for prolonged periods in some patients, even after therapy discontinuation. IFN should be considered as election treatment even in young patients, for its lack of leukemogenic and teratogenic effects.

0382

IS MISCARRIAGE IN ESSENTIAL THROMBOCYTHEMIA (ET) A PROGNOSTIC FACTOR FOR THROMBOSIS?

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Background. Essential thrombocythemia (ET) is a myeloproliferative neoplasm typical of median age with an increased risk of vascular complications. However, ET is also observed in young adults and major thrombotic events may occur also in these patients, who are predominantly women; some of them are diagnosed at childbearing age and their pregnancies have an increased risk of complications represented mainly by fetal loss. Thrombotic occlusion of placental circulation is considered an important mechanism of miscarriage. Relation between thrombosis and JAK2V617F or pregnancy complications and JAK2V617F is strongly suspected. **Aim and Scope.** We retrospectively studied a large cohort of young females with ET to evaluate if pregnancy complications and thrombotic events are related. **Materials, Methods and Patients.** Our study includes 57 consecutive pregnancies (age at conception 32.5±5.4 , range 23-41 years) in 34 females affected by ET (age at diagnosis 29.4 ± 5.8 years, platelets count 851 ±350 x 10^9/L). JAK2V617 mutation was searched with allele specific PCR. Statistical analysis was performed by X^2 test.**4. Results.** Evaluation of JAK2 gene was available in 24 females and 13 (54%) were mutated. Thirty-six (64%) uneventful deliveries were observed, while 21 (36%) pregnancies had a negative outcome (13 first trimester abortions and 8 advanced fetal loss). 27 pregnancies (13 uneventful and 14 fetal loss) (67.5%) occurred in JAK2V617F positive females and 16 (13 uneventful and 3 miscarriages) (32.5%) in JAK2 wild-type (p=0.03).Ten females at mean age of 37.5 ± 5.7

years (30 - 47) had a thrombotic complications: 3 arterial thrombosis (1 myocardial infarction, 1 stroke and 1 intestinal infarction), in all cases at least 10 years after pregnancy and 7 unusual veins thrombosis (1 cerebral, 2 portal and 4 supra-hepatic veins) which occurred in post-partum or no more than two years after delivery. Nine out of the 10 females (90%) with thrombosis had at least one complicated pregnancy.The correlation between pregnancy outcomes and thrombotic events is summarized in the Table 1. **Conclusions.** *JAK2*V617F mutation is confirmed as a negative prognostic factor for pregnancy complications. Our data show that thrombosis occur more frequently in patients who have already had negative pregnancy outcomes. Knowing that a previous thrombosis is a validated risk factor for further cardiovascular event and that placental thromboses have a basic role in pregnancy failures, we suggest that fetal loss should be considered as a thrombotic risk factor for stratification of ET patients. Prospective studies are need to confirm our hypothesis.

Table 1.

	Thombotic complications	No Thombosis	Total	p
Uneventful delivery	9	27	36 (64%)	0.01
Fetal loss	12	9	21 (36%)	

0383

RUXOLITINIB IN CLINICAL PRACTICE FOR THERAPY OF MYELOFIBROSIS: SINGLE USA CENTER EXPERIENCE AFTER FDA APPROVAL

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Background. Ruxolitinib was approved for commercial use for the therapy of intermediate and high risk myelofibrosis (MF) in the USA in November 2011. **Aims.** We sought to assess the initial clinical experience with the use of Ruxolitinib (outside of the clinical trial setting) at our high-volume MPN program. **Methods.** Patients with MF prescribed Ruxolitinib at our center within the first three months after FDA approval were included in this analysis. Baseline disease information, treatment history, dynamics of Ruxolitinib dosing, impact on cytopenias, splenomegaly, and symptoms were assessed. Results from the first four months of therapy are discussed in this abstract. Future results will be presented at the conference. **Results.** A total of 22 patients with MF (post-ET MF (n=6), post-PV MF (n=6) and primary MF (n=10)) began Ruxolitinib between November 2011 and January 2012. Seventeen patients carried the JAK2V617F mutation. The average spleen size prior to starting was 12 cm (0-22 cm). DIPSS-Plus scoring included patients with intermediate-1 risk (n=9), intermediate-2 risk (n=7), and high risk (n=6). Twenty-one patients had received previous therapies with hydroxyurea (68%), anagrelide (22%), interferon-alpha (18%), erythropoietin (13%), JAK-2 experimental agents (13%), danzol (9%) or immunomodulatory agents (9%). Patients had been diagnosed with MF an average of four years prior to initiating Ruxolitinib (0.2-17 years). Initial symptoms included fatigue (63%), nightsweats (59%), abdominal pain (50%), weight-loss (50%), pruritis (22%), early satiety (13%), bone pain (13%) and fevers (9%). The average hemoglobin was 10.9 g/dL (7.6-13.8). Platelet counts averaged 288 x 10^9 (32-844). Four patients had platelet counts less than 100 x 10^9; two patients had values less than 50 x 10^9. Fourteen patients were concurrently receiving MF therapy at the time Ruxolitinib was initiated. Sixteen patients began therapy at 20 mg BID with the remaining starting at a lower dose due to baseline cytopenias. Four patients have undergone dose changes for various symptoms including anemia, thrombocytopenia, hyperkalemia, fatigue and dizziness. One patient discontinued the medication for worsening transfusion-dependent anemia. Two patients required the addition of anagrelide or hydroxyurea for platelet counts >1,000 x 10^9/L. By week three, data from 17 patients was available for review. Six patients had been seen in follow-up and described a decrease in weight loss (n=4), fatigue (n=3), night sweats (n=2), abdominal pain (n=2) and pruritis (n=2). Five had marked reduction of their splenomegaly >5 cm, four of which were non-palpable. By week 4, nine patients had experienced a decrease in hemoglobin values, five of which had more than a 2 g/dL decrease and two requiring a dose reduction. Ten patients had experienced a reduction in platelet counts but all values remained above 100 x 10(9)/L.**Conclusions.** Randomized controlled studies have proven Ruxolitinib's efficacy in reducing debilitating symptoms, splenomegaly, and quality of life in MF patients. This study represents the first evaluation of Ruxolitinib use in clinical practice after FDA approval. Ruxolitinib use in clinical practice appears to mirror the controlled environment of recent clinical trials.

0384

BASELINE DATA FROM A MULTICENTER, PEDIATRIC DISEASE REGISTRY STUDY IN ESSENTIAL THROMBOCYTHEMIA

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Background. Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by an overproduction of platelets. It is associated with an increased risk of thrombohemorrhagic complications and may evolve into myelofibrosis or acute leukemia. Although the median age of presentation is 60-65 years in adults, a significant number of cases have been described in children. However, limited information is available regarding disease complication rates, management approach and treatment outcomes in a pediatric population. **Aim.** The objective of the European pediatric disease registry study (ELLIOT STUDY) is to observe the disease characteristics and management of ET in a pediatric population. Baseline characteristics are described from the most recent data-cut. **Methods**. Children aged 6 to <18 years with ET, were enrolled into the ELLIOT study, after informed consent was obtained. This study is sponsored by Shire Pharmaceuticals. Data were collected at study enrollment and will continue to be collected at all routine clinic visits, for the study observation period (up to March 2016). At least 60 patients are planned to be enrolled into the study by March 2013. **Results.** A total of 51 patients recruited from 26 sites across 6 countries are currently included in the study. Data are described for 26 patients, 61.5% females (n=16) and 38.5% males (n=10), included in the data-cut taken in September 2011.Laboratory and clinical data are summarized in Table 1, comparing features at diagnosis and at study enrollment.

Table 1. Laboratory and clinical data comparing features at diagnosis and at study enrollment.

	At diagnosis	At enrollment
Age (years) Mean (SD)	9.1 (±4.9)	13.9 (±3.3)
Platelets (10^9/L) Median (range)	1251 (1.2–3762)	763 (1.0–2897)
Hb (g/dL) Median (range)	13.1 (10.7–14.4)	13.5 (11.8–15.1)
Hct Median (range)	39.1 (35–43.3)	40.6 (35.4–42)
WBC (10^9/L) Median (range)	11.8 (6.9–14270)	9.1 (5.4–13.6)
Symptoms and complications		
Asymptomatic; n ° (%)		10 (38.5)
Abdominal pain, tiredness, upper respiratory symptoms; n ° (%)		1 (3.8)
Headache; n ° (%)		11 (42.3)
Dizziness; n ° (%)		2 (7.7)
Paresthesia; n ° (%)		2 (7.7)
Foot and hand pain; n ° (%)		1 (3.8)
Nose bleeds and bruising; n ° (%)		1 (3.8)
Photophobia; n ° (%)		1 (3.8)
Thrombohemorrhagic event; n ° (%)		2 (7.7)
Twitching; n ° (%)		1 (3.8)
Splenomegaly; n ° (%)		1 (3.8)
Venous thrombosis; n ° (%)		1 (3.8)

Hb – Hemoglobin; Hct – Hematocrit; WBC – White blood cells

The most common complication observed was headache, experienced by 42.3% of patients (n=11). *JAK2* status was reported in only 4 patients (V617F positive, n=1).Bone marrow evaluation was performed in 17 cases by aspirates (65.4%); cytogenetic analysis was performed in 16 patients (61.5%), of which 1 was abnormal. In 9 patients trephine biopsies were performed: 7 had abnormal megakaryocytes, and fibrosis assessment in 7 patients showed grade 1 in 6 patients and grade 2 in 1 patient.The majority of patients (65.4%, n=17) did not receive cytoreductive treatment at or prior to study enrollment. Of the 9 patients who received cytoreductive treatment, a similar proportion had previously been treated with anagrelide (n=6) and/or hydroxycarbamide (n=5). Only four patients were still receiving cytoreductive treatment at study enrollment (all anagrelide). The median duration of anagrelide and hydroxycarbamide treat-

ment was 668 days (range, 185-2174) and 337 days (range, 32-1382), respectively. In the whole patient cohort, the most common concomitant medication at or prior to study enrollment was aspirin (38.5%, n=10). **Summary and Conclusions.** This study provides valuable epidemiological data concerning the clinical presentation and the management of children with ET, as observed in European pediatric hematological centers. The results show that while most patients do not receive cytoreductive treatment, approximately one-third of children are treated at some stage with anagrelide and hydroxycarbamide in similar proportion, even in the absence of major thrombohemorrhagic complications. Further information is required to understand factors affecting treatment choice and clinical management of ET in pediatric patients. This study highlights the need for guidance in the treatment of ET for pediatric patients. The study is ongoing.

0385

ANEMIA AND THE USE OF ERYTHROPOIETIC-STIMULATING AGENTS (ESAS) WITH RUXOLITINIB IN THE COMFORT-II STUDY

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Background. Ruxolitinib is a potent and selective JAK1/2 inhibitor approved in the US based on results of the phase 3 COMFORT studies. Ruxolitinib demonstrated rapid and durable reductions in splenomegaly and improved disease-related symptoms and quality of life. Consistent with ruxolitinib's known mechanism of action, anemia was one of the most frequently reported adverse events (AEs) but was generally manageable and led to discontinuation in only 1 of 146 patients. In clinical practice, anemia can sometimes be managed with ESAs, which promote red blood cell proliferation via cytokine receptors that signal through the JAK pathway. Because these agents act upstream of ruxolitinib inhibition, it is important to determine their effect on the safety and efficacy of ruxolitinib and vice versa. **Aims.** This post hoc analysis evaluated the safety and efficacy of ruxolitinib in patients receiving concomitant ESAs in COMFORT-II. **Methods.** COMFORT-II is an open-label, randomized (2:1), multicenter, phase 3 study comparing ruxolitinib with best available therapy (BAT) in patients with primary MF, post-polycythemia vera MF, and post-essential thrombocythemia MF. Use of ESAs, though not prohibited, was discouraged for patients receiving ruxolitinib because these drugs can increase spleen size, which could confound efficacy analyses. **Results.** ESA use was reported for 9 of the 146 patients receiving ruxolitinib (+ESA group; darbepoietin-alpha, 1.4%[n=2]; epoetin-alpha, 4.8%[n=7]; median exposure, 22.1 weeks). Overall, the median duration of ruxolitinib exposure was 51.4 weeks, and median dose intensity was similar in the +ESA group compared with the non-ESA group (39.2 vs 30.0 mg/day). The mean change in spleen volume from baseline at week 48 was similar in both groups (+ESA, -29.8%; non-ESA, -30.1%); 44.4% (4/9) of the +ESA group and 27.0% (37/137) of the non-ESA group achieved ≥35% reduction in spleen volume from baseline at week 48. The mean number of packed red blood cell units received per month while on treatment was similar in both groups (+ESA, 0.78; non-ESA, 0.86). AEs reported in both groups were similar; serious AEs (SAEs) were reported for 4 patients in the +ESA group. SAEs determined to possibly be related to ruxolitinib (respiratory infection and decreased general condition) occurred in 1 patient who discontinued the study; this patient died from respiratory infection. At baseline, the +ESA and non-ESA groups had median creatinine levels of 70.2 and 75.4 µmol/L, respectively. Median changes from baseline at week 48 were similar for reticulocytes (+ESA, -13.6%; non-ESA, -9.1%) and hemoglobin (+ESA, -5.8%; non-ESA, -5.7%). The +ESA group had higher median levels of erythropoietin at baseline (39.0 vs 7.0 pg/mL) and a larger increase at week 48 (485.0% vs 185.2%). **Conclusions.** Ruxolitinib provides significant reduction in spleen size compared with BAT, and in these analyses, concomitant ESA use did not appear to affect these reductions. No substantially different AEs were reported in patients receiving ruxolitinib and ESAs. Concomitant use of ESAs may have allowed for an increased dose of ruxolitinib. Because the use of ESAs is common in clinical practice, further analyses are warranted.

0386

RED CELL MASS DETERMINATION IN PATIENTS WITH CLINICALLY SUSPECTED DIAGNOSIS OF POLYCYTHEMIA VERA OR ESSENTIAL THROMBOCYTHEMIA

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Background. WHO criteria for diagnosis of polycythemia vera (PV) include the presence of hemoglobin >16.5 g / dL in women, 18.5 g / dL in men or other evidence of increased red cell volume. In patients with clinically suspected diagnosis of essential thrombocythemia (ET) or PV who do not reach the WHO Hb level for PV diagnosis, the indication of red cell mass assessment is not well established. **Aims.** To identify the cut-off for hemoglobin (Hb) or hematocrit (Hct) that indicate the need for a red cell mass study in patients with a presumptive diagnosis of PV or ET. **Methods.** Red cell mass, by isotopic ^{51}Cr, was determined in 179 patients (91 women, 88 men) with clinically suspected diagnosis of ET or PV and a Hct higher than 0.42 L/L (females) or 0.45 L/L (men). Receiving operating characteristic (ROC) curves were performed to evaluate the diagnostic accuracy of Hb and Hct in order to distinguish a normal or an increased red cell mass. We determined the specificity and sensitivity of each Hb and Hct value. The cut-off was selected doing the sensitivity prevailing over the specificity in order to reduce the number of false negative cases. **Results.** An increased red cell mass was documented in 114 patients (64 males, 50 females). The ROC curves showed a greater area under the curve (AUC) for Htc than for Hb [AUC for Htc = 0.92 (95%CI: 0.88-0.96) and AUC for Hb= 0.88 (95%CI: 0.84-0.93)] with similar results being observed when patients were stratified by gender. The best value of Hct to indicate a red cell mass study was 0.48 L/L in women (specificity 73%, sensitivity 94%) and 0.50 L/L in men (specificity 75%, sensitivity 87.5%). Lowering the Htc threshold to 0.48 L/L in males increased the sensitivity up to 95% with only 3 false negative cases and an acceptable number of false positives (14 cases with Htc > 0.48 L/L out of 24 cases with normal red cell mass). The Hb cut-off points proposed in the WHO classification for diagnosis of PV showed a high specificity but a low sensitivity, resulting in a great number of false negatives (26 women and 27 men) that would be misdiagnosed as essential thrombocythemia if a red cell mass study was not performed. **Conclusions.** In patients with clinically suspected diagnosis of ET or PV who do not reach WHO defined Hb values to establish the diagnosis of PV, a red cell mass study should be performed when the Hct is higher than 0.48 L / L.

0387

CLINICAL SIGNIFICANCE OF CLONALITY ASSESSMENT IN JAK2V617F-NEGATIVE ESSENTIAL THROMBOCYTHEMIA

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Background. JAK2V617F-negative essential thrombocythemia (ET) is a heterogeneous disease including patients with clonal hematopoiesis demonstrated by mutations in different genes or by X-chromosome inactivation patterns (XCIP) and others without molecular markers of clonality. It is unknown if patients with evidence of clonality have a different clinical outcome than those in whom clonal hematopoiesis cannot be demonstrated. **Aim.** To evaluate the clinical significance of clonality demonstration in a cohort of JAK2V617F-negative ET patients. **Methods.** In this study, 73 JAK2V617F-negative cases out of 186 subjects consecutively diagnosed with ET, in a single institution, were assessed for clonality by means of the *HUMARA* assay and analysis of the mutational status of *MPL* (exon10), *TET2* (whole coding sequence) and *ASXL1 (*exon 12). **Results.** Clonality by *HUMARA* assay was demonstrated in 21 patients (46%) out of 46 assessable females whereas mutations in *MPL*, *TET2* and *ASXL1* were observed in 7, 4, and 2 cases out of 73, respectively. The detection of any mutation (*MPL*, *TET2* or *ASXL1*) was more frequently observed in patients with clonal *HUMARA* (8 mutations out of 20 clonal cases) than in those with polyclonal hematopoiesis (1 case out of 23 polyclonal cases) being the difference statistically significant (p=0.007).With a median follow-up of 8 years, death, thrombosis, bleeding and disease transformation were registered in 7, 10, 8, and 6 patients (5 myelofibrosis, 1 myelodisplasia), respectively. No differences in thrombosis, bleeding or survival were observed according to the presence of clonality assessed either by *HUMARA* assay or mutational status. The probability of disease transformation at 10 years was higher in patients showing clonal hematopoiesis by *HUMARA* analysis (35% versus 0% in patients with polyclonal hematopoiesis, p< 0.004), and in those with presence of mutations in either *MPL*, *TET2* or *ASXL1* (64% versus 2% in patients without mutations, p<0.001). **Conclusions.** In conclusion, patients with JAK2V617F-negative ET who show clonal hematopoiesis demonstrated by the *HUMARA* assay or by the presence of mutations in the *MPL*, *TET2* and *ASXL1* genes, have a higher probability of disease transformation. **Acknowledgements**This study was supported in part by grants EC10-136, FISPI10/01807 and AECC Catalunya 2011

Anemia and transfusion

0388

EFFICACY AND SAFETY OF DEFERASIROX IN PATIENTS WITH IRON OVERLOAD SECONDARY TO HEREDITARY HEMOCHROMATOSIS: PRELIMINARY RESULTS OF A 1-YR PROSPECTIVE STUDY

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Background. Hereditary hemochromatosis (HH) is one of the most common genetic diseases, and may be associated with excessive absorption of dietary iron in some cases. If untreated, progressive iron accumulation in key organs leads to toxicity, resulting in tissue damage and organ dysfunction. Iron overload (IOL) can be readily managed in most patients by therapeutic phlebotomy. However, patients with underlying anemia and/or difficult venous access may not tolerate this treatment. In addition, compliance may be variable over time as a result of the inconvenience of frequent visits and the discomfort associated with the procedure. Clinical studies have demonstrated the efficacy, safety, and tolerability of deferasirox (DFX) in patients with a variety of conditions associated with transfusional IOL. Studies reporting iron chelation therapy in patients with primary (nontransfusional) IOL are limited, but data are encouraging. Aims and Methods : DFX at the starting dose of 10 mg/kg/day was administered orally for 1 year in patients with HH (C282Y/C282Y, C282Y/H63D or H63D/H63D gene mutation) and IOL (defined as transferrin saturation [TS] ≥45% and serum ferritin [SF] level ≥ 500 µg/L confirmed at two visits and liver iron concentration (LIC) by MRI ≥ 5 mg Fe/g dw at study entry) who were unable or unwilling to comply with a regimen of phlebotomy. The study objectives were to evaluate the changes in TS, SF and LIC levels and the efficacy (% of SF reduction from baseline and/or achievement of SF < 300 µg/L) and safety of DFX. Efficacy was assessed monthly by measuring changes from baseline in TS and SF levels. Safety was evaluated monthly based on the incidence and type of adverse events and changes in laboratory parameters, including serum creatinine and liver enzyme. LIC by MRI was measured at baseline and after 1-year of treatment or when SF was < 300 µg/L. **Results.** A total of 10 adult patients were included and all of them completed the 1-yr treatment period. Median age was 52.0 years; 60% female, 100% caucasian, 70% with C282Y/C282Y HFE gene mutation. Median SF levels(µg/L) reduced significantly at 12 months compared to baseline (from 1608.5 to 300.0, p<0.001). Median LIC significantly dropped at 12 months compared to baseline (from 14.5 to 5.5, p<0.001). The median time to achieve a SF reduction ≥ 50% compared to baseline was 7.53 months. The most common drug-related AEs were mild, transientdiarrhea (5 patients) and nausea (2). No patient experienced increases in serum creatinine that exceeded the upper limit of normality. **Conclusions.** Our preliminarydata confirm that deferasiroxis effective in reducing body iron burden and well tolerated in patients with HH, with a clinically manageable safety profile when used at appropriate dose, and could be a safe treatment option to phlebotomy in selected patients.

0389

I.V. IRON USAGE IN CHEMOTHERAPY-INDUCED ANEMIA: AN OBSERVATIONAL, PAN-EUROPEAN STUDY

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Background. Intravenous (I.V.) iron supplementation is superior over oral or no iron for the treatment of chemotherapy-induced anemia, a frequent comorbidity in cancer patients. International guidelines recommend I.V. iron to prevent/reduce blood transfusions and doses of erythropoiesis-stimulating agents (ESAs). However, recommendations on the optimum I.V. iron treatment schedule are still lacking. **Aims.** This study evaluated current I.V. iron treatment practice and its link to anti-cancer treatment. **Methods.** Onco-hematologists in France, Germany, Spain, UK and Switzerland (March-May 2011) reported data on patient demographics, hemoglobin (Hb) levels, iron parameters, and therapies of anemic cancer patients on chemotherapy who had received their first I.V. iron treatment and had available records for at least 3 months prior and after this treatment. Results are shown as percentages of all patients. **Results.** 32 Onco-hematologists reported 101 cases of which 87% had solid or hematologic tumours (68% stage IV) and 13% myelomas (54% ISS III). In 68%, the first iron infusion took place within one year after cancer diagnosis and in 41% on the day of anemia diagnosis. Anemia assessment intervals were ≤4 weeks in 94%. Before first I.V. iron treatment, 62% of patients had Hb <9.0 g/dL (96% tested), 50% had serum ferritin ≤30 µg/L (82% tested) and 60% a transferrin saturation <20% (25% tested). In 46%, I.V. iron was given as monotherapy (Spain 77%, Switzerland 71%), additional ESA or oral iron was given in 7% each. 25% had a transfusion at any time during the observation period (most frequently in UK 48%); 14% were switched from blood transfusion(s) to I.V. iron. Another 14% were switched from I.V. iron to ESA.Reasons for initiation of I.V. iron treatment were lack of response (20%) or intolerance (12%) to prior treatment. Mean Hb levels decreased from 9.6 to 8.7 g/dL during 3 months prior the first I.V. iron administration, and improved to 10.2 g/dL in the following 3 months when 61% received I.V. iron as sole anemia treatment. Mean total I.V. iron dose per patient was 732 mg. 49% received more than one I.V. iron dose and those with initial iron doses ≥500 mg (22%) showed a trend for a longer mean interval until the second I.V. iron dose (35.4 vs. 29.2 days) compared to those with initial doses <500 mg. Use of initial iron doses below or above 500 mg was independent of baseline Hb. Taxane-based chemotherapies were given in 28% of patients, anthracycline-, cisplatin- and oxaliplatin-based therapies in 19, 18 and 15%, respectively. In 58%, chemotherapy and I.V. iron were given within 1-3 days, although most physicians (85%) perceived the treatments as not connected. **Conclusions.** Practice in I.V. iron use, particularly the dosing strategy, varies considerably between countries, suggesting a need for updating anemia treatment guidelines with iron-specific aspects. Earlier initiation of I.V. iron treatment may prevent the decrease of already low Hb levels in patients with chemotherapy-induced anemia. Iron doses ≥500 mg may reduce treatment frequency. TSAT is still underused as iron status parameter. Intravenous iron was given in combination with most common chemotherapies.

0390

POST-HOC ANALYSIS OF HEMATOLOGIC RESPONSES INCLUDING TRANSFUSION INDEPENDENCE IN IRON-OVERLOADED APLASTIC ANEMIA PATIENTS TREATED WITH DEFERASIROX

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Background. Reports of iron chelation therapy in aplastic anemia (AA) patients have mainly focused on efficacy and safety of excess iron removal. The 1-year, EPIC study enrolled 116 iron-overloaded AA patients; deferasirox significantly reduced iron overload as assessed by serum ferritin levels. As iron overload has a suppressive effect on erythroid progenitors and may increase transfusion requirements, it is of interest to evaluate hematologic responses with iron chelators in AA. **Aims.** To evaluate hematologic parameters in iron-overloaded AA patients treated with deferasirox. **Methods.** EPIC study design and inclusion/exclusion criteria have been previously described (Cappellini *et al. Haematologica* 2010). For this *post-hoc* analysis, UK treatment guideline criteria for AA diagnosis were used (2 or 3 of the following: hemoglobin [Hb] <100g/L, platelets <50x10^9/L, neutrophils <1.5x10^9/L) with hematologic response criteria reported by Camitta (*Acta Haematol* 2000). Bone marrow and reticulocyte data were not recorded in EPIC, hence patients were classified as 'severe' AA based on fulfillment of platelet/neutrophil criteria. For severe AA patients, hematologic response was defined as: no response=still severe; partial response=transfusion independence, and no longer meeting criteria for severe disease; complete response=normal Hb for age, neutrophil count >1.5x10^9/L and platelet count >150x10^9/L. For 'non-severe' AA patients, response was defined as: no response=worse/not meeting criteria for partial/complete response; partial response=transfusion independence (if previously dependent), or doubling or normalization of at least one cell line, or increased Hb >3 g/dL if initially <6g/dL, or increased neutrophils >0.5x10^9/L if initially <0.5x10^9/L,

or increased platelets >20x10^9/L if initially <20x10^9/L; complete response=same as severe. Each criterion was confirmed with no measure within 28 days that disproved the response. Transfusion independence was defined as at least one 8-week period without transfusion in patients transfusion-dependent during the study. Patients with no transfusions in the year prior to study entry were excluded from transfusion responders. **Results.** Evaluable hematologic parameters were recorded in 72/116 (62.1%) iron-overloaded AA patients; nine (12.5%) and 63 (87.5%) with severe and non-severe AA, respectively. Twenty-four (33.3%) patients received deferasirox without concomitant immunosuppressive treatment (IST) and 48 (66.6%) patients received ≥1 concomitant IST during the study. Partial responses were observed in 30 (41.7%) patients (Figure); 11/24 (45.8%) patients without and 19/48 (39.6%) with concomitant IST. All 11 patients achieving a partial response without concomitant IST had non-severe AA and became transfusion-independent (11/24, 45.8%); two patients had an additional platelet response and one patient achieved a platelet and Hb response. Most patients achieving a partial response with concomitant IST also became transfusion-independent (15/19, 78.9%); two patients had an additional neutrophil response (one patient with non-severe and one with severe AA), one had an additional platelet and Hb response (non-severe AA) and one had an additional neutrophil, platelet and Hb response (severe AA). **Summary and Conclusions.** Deferasirox may improve hematologic parameters in AA patients if it is administered concomitantly with IST or not. The most common response was transfusion independence, as also observed in patients with other bone marrow failure conditions (Guariglia *et al. Leuk Res* 2011). Additional studies to confirm these results and to clarify mechanisms are required.

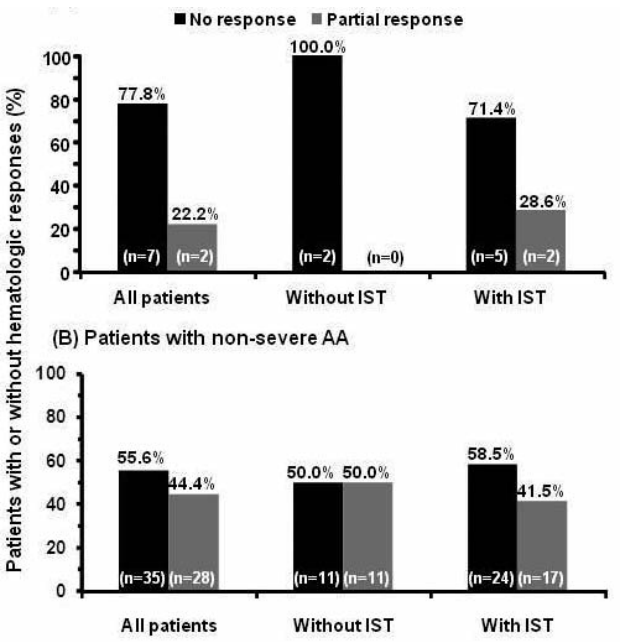

Figure 1. Hematological responses with or whitout IST in patients with (A) severe AA and (B) non-severe AA (A) patients with severe AA.

0391

PHENOTYPE OF HETEROZYGOUS TMPRSS6 DEFECTS IN 4 DUTCH FAMILIES SUGGESTS THAT IRON REFRACTORY IRON DEFICIENCY ANEMIA IS A CO-DOMINANT DISEASE

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Background. Iron Refractory Iron Deficiency Anemia (IRIDA) is a rare hereditary disease characterized by microcytic hypochromic anemia with low transferrin saturation (TSAT), not or only partially responsive to oral iron. The genetic substrate of IRIDA is the *TMPRSS6* gene (transmembrane protease serine 6), encoding the protein matriptase-2. *TMPRSS6* defects lead to increased levels of hepcidin, inappropriate for the low body iron status, resulting in decreased intestinal iron absorption and iron utilization by degradation of the cellular iron exporter ferroportin.Inheritance of IRIDA is still under debate. Most data from the literature indicate that IRIDA is an autosomal recessive disease. However, anecdotic reports describe heterozygous pathogenic TMPRSS6 mutations resulting in clinical apparent disease.In this abstract we report on 4 index cases with heterozygous pathogenic *TMPRSS6* defects resulting in mild IRIDA phenotypes. Family screening of the index cases showed only TMPRSS6 heterozygotes without the IRIDA phenotype. These data support the hypothesis that IRIDA is a co-dominant disease with variable penetrance. **Aims.** Description of inheritance and clinical and biochemical spectrum of IRIDA in index cases and relatives heterozygous for a pathogenic *TMPRSS6* defect. **Methods.** Case series**. Results.** Four index cases presented with mild microcytic hypochromic anemia with low transferrin saturation (TSAT), not or only partially responsive to oral iron supplementation. In 3 out of 4 index cases serum hepcidin, analysed by time-of-flight mass spectrometry, was inappropriately high relative to the low TSAT, suspicious of IRIDA (range TSAT/hepcidin ratio 0.3 to 1.0 %/nM; reference ranges men 1.7-256%/nM, premenopausal women 2.0-330%/nM, postmenopausal women 1.5-73.4%/nM; www.hepcidinanalysis.com). In the other index case the TSAT/hepcidin ratio was 2.1%/nM.DNA sequencing of the coding regions of the *TMPRSS6* gene between 2009 and 2012 showed a heterozygous pathogenic defect in all index cases (p.Cys702Phe, n=2; c.863+1G>T, in the 5' splice site of intron 7, n=1; p.Gly442Arg, n=1) Family screening of the 4 index cases was performed and showed 6 first degree relatives with the heterozygous pathogenic *TMPRSS6* defect.All these 6 heterozygous relatives were asymptomatic with Hb, MCV and TSAT/hepcidin ratio within reference ranges. This heterogeneity in phenotype could not be explained by modulating *TMPRSS6* polymorphisms cis or trans of the affected allele. Also there was no evidence for environmental factors influencing the biochemical /clinical penetrance in the affected index cases, such as hypermenorrhoe or chronic inflammation. **Summary and Conclusions.** We conclude that IRIDA is a co-dominant disease with variable clinical penetrance due to still unknown genetic and/or environmental factors, indicating that the inheritance of IRIDA still needs elucidation.

0392

ENDOTHELIAL DERIVED-MICROPARTICLES AS MARKER OF ENDOTHELIAL INJURY IN ABO - HEMOLYTIC DISEASE OF THE NEWBORN

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Background. ABO blood group incompatibility between mother and the fetus is currently the most frequent cause of hemolytic disease of the newborn (HDN). The ABO antigens are expressed on the surfaces of red cells and many other tissues, including vascular endothelium. Circulating endothelial microparticles (EMPs) are complex vesicular structures shed from activated or apoptotic endothelial cells and play a remarkable role in coagulation, inflammation, endothelial function, and angiogenesis contributing to the progression of vascular diseases. **Aims.** This work aimed to study circulating EMPs in ABO-HDN as a marker of endothelial activation , to examine the hypothesis of possible endothelial injury in neonates with ABO blood group incompatibility, and its relation to the occurrence and severity of hemolytic disease. **Methods.** Sixty-five full term neonates with HDN; 45 with ABO incompatibility and 20 with Rh incompatibility were compared to 20 healthy neonates with ABO and Rh matched mother and infant blood groups (controls). All neonates were studied stressing on blood grouping, markers of hemolysis ,and investigations were done to exclude other causes of neonatal hemolytic disease. Circulating EMPs ($CD144^+$) were measured by flow cytometry , before and after therapy (phototherapy and/or exchange transfusion). Patients with HDN were further subdivided according to the age-specific total serum bilirubin nomogram and disease severity into 3 subgroups; mild, moderate and severe HDN. **Results.** There was no significant difference between ABO and Rh HDN patients regarding birth weight,gestational age,hemoglobin,LDH, and bilirubin , however neonates with Rh HDN had higher reticulocytic count levels (p=0.008), and all (100%) had positive direct and indirect antiglobin test , compared to 17.8% and 15.6% in ABO HDN (p<0.001 and p=0.067,respectively). Pre-therapy EMPs levels were elevated in ABO and Rh HDN compared to controls (p<0.001, and p=0.02, respectively) ,being significantly higher in ABO compared to Rh HDN (p=0.02) . In ABO HDN , pre-therapy EMPs levels were significantly elevated in severe disease compared to moderate and mild disease (p<0.001),meanwhile, no significant difference was observed in EMPs levels in neonates with Rh-HDN in relation to disease severity (p=0.09) . Neonates with severe ABO-HDN had the highest pre-therapy EMPs compared to Rh-HDN (p<0.001). Multilinear regression analysis revealed that hemoglobin, reticulocytic count ,LDH and indirect bilirubin were independently correlated to pre-therapy EMPs levels in ABO HDN (r^2=0.834, p<0.001) , while in Rh HDN only hemoglobin and LDH were strongly significant (r^2=0.877, p<0.001). Post-therapy EMPs levels were significantly decreased after phototherapy as well as after exchange transfusion compared to pre-therapy levels (p<0.001) ,observed in both ABO and Rh-HDN groups , however, the decline in EMPs levels was remarkably evident

after exchange transfusion in neonates with severe ABO-HDN (p<0.001). **Conclusions.** We suggest that elevated EMPs in ABO-HDN may reflect an IgG-mediated endothelial injury parallel to the IgG- mediated erythrocytes destruction and thus could serve as a surrogate marker of vascular dysfunction and disease severity in those neonates. The proof of this concept remains to be fully elucidated and poses important challenges for risk stratification and therapeutic response.

0393

A MAGNETIC RESONANCE IMAGING ASSESSMENT OF CARDIAC AND LIVER IRON LOAD IN PATIENTS WITH HEMOGLOBINOPATHIES, MYELODYSPLASTIC SYNDROMES OR OTHER ANEMIAS TREATED WITH DEFERASIROX (MILE - CICL670AAU01)

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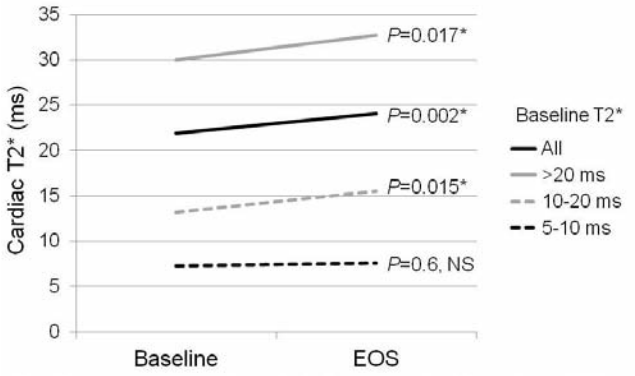
Background. Cardiac failure is the leading cause of death in patients with thalassemia major. However, the introduction of new monitoring techniques, such as cardiac iron Magnetic Resonance Imaging (MRI) may lead to the improvement of patient care and prolong survival. The examination of the effect of iron chelators such as deferasirox on cardiac iron reduction is crucial in the development of treatment protocols, while the clinical consequences of cardiac iron accumulation in myelodysplastic syndromes (MDS) is still controversial. **Aims.** The primary objective of this study is to investigate the effect of single agent deferasirox on cardiac iron levels and cardiac function in a cohort of patients receiving regular red blood cell transfusions. **Methods.** Patients with transfusional siderosis were enrolled in this study and received deferasirox up to 40 mg/kg/day for 53 weeks. The primary endpoint was to investigate the change in cardiac T2* at 53 weeks compared to baseline (power and sample size calculation based on previously published literature). Secondary efficacy endpoints included change in left ventricular ejection fraction (LVEF), liver iron concentration (LIC) by R2-MRI and serum ferritin. All analyses were performed for all subjects in the full analysis population that included patients with hemoglobinopathies and MDS. Informed consent was obtained from all patients.



Results are presented as the adjusted means on back-transformed T2* values after the analysis model. *Statistically significant. NS: non-significant. EOS: end of study.

Figure 1. Change in cardiac T2* after 1 year.

Results. Results were available for 40 patients with hemoglobinopathies (36 thalassemia major, 4 sickle cell disease) and 6 patients with MDS (inferential statistics, all 46 patients with baseline and post-baseline cardiac T2* values). An overall increase in cardiac T2* from 21.9 to 24.1 ms was observed (10% increase, 95% CI=4 to 16%, *P*=0.002). Further analysis was performed based on T2* baseline values: 5-10 ms, no significant change (n=5; 7.3 to 7.6 ms; *P*=0.6); 10-20 ms, improvement of 18% (n=9 from 13.2 to 15.5 ms; *P*=0.015); >20 ms, increase of 9% (n=32; 30 to 32.7 ms; *P*=0.017). Descriptive statistics showed that in the subgroup of cardiac T2* 5-10 ms, patients had either received mean doses of deferasirox <30 mg/kg/day, or had a baseline LIC >25 mg Fe/g dry tissue. All patients in the MDS subgroup had baseline cardiac T2* >20 ms (descriptive analysis, median 30 ms; 24.4 to 33.2 ms), with a non-statistically significant increase in cardiac T2* after one year (12%; 29.3 to 32.7 ms, *P*=0.18). Statistically significant reductions in LIC were observed for the overall patient population (9.7 to 7.4 mgFe/g dry tissue *P*=0.037), especially for those with baseline LIC >7 mgFe/g dry tissue (20.2 to 15.1 mgFe/g dry tissue; *P*=0.003). A statistically significant reduction in serum ferritin of 7.6 µg/L/week was observed in the overall patient population (*P*=0.009). Safety marker analyses were consistent with previous reports. Results on LVEF will be presented. **Conclusions.** These results showed that once-daily deferasirox administered over 1 year induced a statistically significant increase in cardiac T2* levels, and a reduction in LIC and serum ferritin. This confirms previous reports showing that single agent deferasirox is an effective therapy for maintenance and reduction of cardiac iron levels, especially for patients with cardiac T2* >10 ms.

0394

FEATURES OF TREATMENT AND PROGNOSIS OF NON-ST ELEVATION ACUTE CORONARY SYNDROME IN PATIENTS WITH IRON DEFICIENCY ANEMIA

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The aim of the study was to determine features of clinical course and prognosis of non-ST elevation acute coronary syndrome in patients with iron deficiency anemia and high risk of bleeding. The authors analyzed the medical histories of 2473 patients admitted to the coronary care department from October 2006 to October 2009 year with diagnosis of non-ST elevation acute coronary syndrome, and 339 conclusions of post-mortem examination. Based on analysis of archival data shows, that the frequency of occurrence of anemia in patients with non-ST elevation acute coronary syndrome is 64,5%. Physicians rarely assess the risk of bleeding in these patients, and therefore antithrombotic therapy is appointed only in 62,6% of cases. In addition, at patients with hemoglobin below 90 g/l antithrombotic therapy is carried out in only 30,7% of cases. The opposite situation is observed with respect to the correction of iron deficiency anemia. Patients with non-ST elevation acute coronary syndrome and low level of hemoglobin are appointed iron preparations in 44,9% of cases, while patients with mild iron deficiency anemia - only in 6,5% of cases. Meanwhile, the incidence of myocardial infarction and mortality of these patients depends on the initial level of hemoglobin, as well as from ongoing antithrombotic therapy. Without it, antithrombotic complications occur in 22,1% of cases in the structure of all fatal events. In addition, demonstrated, that in patients with non-ST elevation acute coronary syndrome the incidence of myocardial infarction and mortality while severe anemia are 97,3% and 78,1% respectively. Proved that the highest frequency of fatal (22,1%) and non-fatal (37,4%) thrombotic complications occurs in patients with non-ST elevation acute coronary syndrome and iron deficiency anemia, which antithrombotic therapy in the hospital was not carried out. In these patients the frequency of hemorrhagic complications is equal to 4,3%. We demonstrated that using of antithrombotic therapy by low-molecular-weight heparin (dalteparin natrium) on the background correction of iron deficiency by aqueous complex of polynuclear iron (III)-hydroxide (Venofer ®), reduces mortality, risk of bleeding, symptoms of iron deficiency anemia and frequency of myocardial infarction development in patients with non-ST elevation acute coronary syndrome

0395

SPLENIC ARTERY EMBOLIZATION FOR THE TREATMENT OF THROMBOCYTOPENIA AND HYPERSPLENISM IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a disease char-

acterized by hemolytic anemia, cytopenias and a high risk of venous thrombosis. Splenomegaly is not common: when it occurs, it is usually the consequence of splenic or portal vein thrombosis. PNH patients who have splenomegaly may also have thrombocytopenia, or neutropenia, or both, which may reflect hypersplenism. The management of this complication is challenging because splenectomy is hazardous in the presence of portal hypertension and it may be complicated by further thrombosis. **Aims.** Aim of this study was to test the safety and efficacy of selective splenic artery embolization (SAE), as an alternative to splenectomy, in patients with PNH who had thrombosis, splenomegaly and thrombocytopenia (TST). Our purpose was to devitalize part of the spleen without incurring in the risks of abdominal surgery. **Methods.** Four PNH patients had: (a) multiple thrombosis involving the portal system, (b) splenomegaly and (c) severe thrombocytopenia (median PLTS 15 x 10^9/L), presumed to be at least in part secondary to hypersplenism; 3 of the 4 patients also suffered from disabling recurrent episodes of abdominal pain. All 4 patients underwent SAE through interventional radiology, whereby branches of the main splenic artery, that are end-arteries distal to the splenic hilum, were occluded, causing infarction of the splenic parenchyma. In order to avoid an abrupt infarction of the entire spleen, which may be complicated by splenic rupture, splenic abscess, pneumonia or sepsis, SAE was carried out in steps, at several weeks or months intervals. **Results.** All patients witnessed a reduction/normalization of the spleen size. The 3 patients who suffered from recurrent episodes of abdominal pain experienced a resolution of the symptoms. Three out of 4 patients had a clinically significant increase in platelet counts: in 1 patient this took place already after the first SAE procedure and in 2 after 3 procedures. The procedures were uneventful, except for a pleural effusion in 1 case. With a follow-up time of 3 to 12 years, in 3 patients the clinical and hematological improvement was long-lasting. In the patient whose thrombocytopenia was not corrected, the reason was clearly bone marrow failure in addition to hypersplenism: this patient subsequently underwent a successful hematopoietic stem cell transplantation. In 1 patient, who was on eculizumab and had evidence of extravascular hemolysis, after SAE we observed, in addition to a remission of her thrombocytopenia, also a significant reduction in transfusion requirement. **Summary.** In conclusion, we have shown that SAE, carried out in sequential steps, is of benefit to patients with PNH who have the TST complex. A clinically significant resolution of pain was seen in all cases and an objective response was documented in terms of spleen size (4/4 cases) and of platelet counts (3/4 cases). In addition, for patients who, on account of the extravascular hemolysis remain transfusion dependent on eculizumab, SAE may be beneficial and probably a preferable alternative to splenectomy.

0396

THE VALUE OF RADIOCHROMATED ERYTHROCYTES IN DEMONSTRATING GASTROINTESTINAL TRACT BLOOD LOSS AS A SILENT CAUSE OF IRON DEFICIENCY

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Background. Occult blood loss from the gastrointestinal (GI) tract, causing iron deficiency often with anaemia, can be diagnostically and therapeutically challenging. This is because stool testing may be negative, endoscopy poorly informative in view of intermittent bleeding while barium studies can potentially overlook superficial lesions. These limitations can be approached using two different radioactive isotopes to first reliably demonstrate the appearance of significant label in the stool and then localise the site anatomically.

Table 1. Summary of demographics and results of radionuclide studies.

Study	Age (years)		Sex		Result		
	Range	Mean	F	M	Positive	Negative	Total
Cr-51 RBC	1.5-81	50	36	21	30 (53%)	27 (47%)	57
Tc-99m RBC	36 -81	65	8	7	12 (80%)	3 (20%)	15

Aims. To demonstrate the usefulness of chromium-51 (^{51}Cr) labeled red blood cell (RBC) in detecting and quantifying blood loss from the GI tract and aiding in timing of Technetium-99 (^{99m}Tc) labeled RBC study, improving the sensitivity of the study to localize the site of GI bleeding. Finally to correlate these findings with clinical outcome. **Methods.** In this retrospective review, records of patients referred for evaluation of possible GI blood loss were reviewed, in each; daily recovery of radiochromium from stool was measured in the whole body counter. In those cases exceeding 50 ml/day, a technetium-99m (Tc-99m) localization study was performed.These studies were correlated with clinical findings. **Results.** 36 female and 21 males (n = 57) participated with institutional and ethics committee approval. In 30 (51 %) the radiochromium result was positive. In 15 of these, 12 (80%) technetium imaging then successfully defined a specific anatomical site: Ten of the latter were evaluable and half diagnosed with small-bowel angiodysplasia **Summary and Conclusions.** This sequential twin isotope method is practical in revealing otherwise silent intestinal haemorrhage. It has good patient acceptability and clinical as well as diagnostic utility in management.

0397

USE OF CAPILLARY BLOOD SAMPLES TO PERFORM CONFIRMATORY TESTS. AN AID FOR AN EARLIER DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS (HS) IN NEONATES AND SMALL INFANTS

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Background. Blood volume required to perform confirmatory tests for HS may be hard or even not feasible to collect in neonates or infants due either to difficulty inherent to the sampling procedure or to the inconvenience of withdrawing large blood volumes in small infants. Moreover, some patients with severe hemolytic anemia may require either red blood cells transfusions or exchange transfusions; in these cases, confirmatory tests must be delayed for some time. As a consequence, etiological diagnosis of hemolytic anemias may be delayed several weeks or months. Although capillary blood sampling is an easy procedure, its use to perform HS laboratory tests has not been reported in the literature. We have separately evaluated the accuracy of capillary blood for hypertonic cryohemolysis (CH), eosin-5'maleimide-flow cytometry (5'EMA-FC), and flow cytometric osmotic fragility (OF-FC), concluding that results showed no difference with the use of venous blood. In this study, we simultaneously performed the three tests. **Aims.** To assess the reliability of capillary blood samples for HS diagnosis. **Methods.** Twenty nine children, aged 2 days to 7 years (median 7 months), with hemolytic anemia or with a relative with HS were studied. Blood samples were collected by digital or heel punction in heparinized microhematocrit tubes. Six tubes (300 µL) per patient were collected for performing all tests. Our reference cut-off values for HS diagnosis are: CH >2.8%; 5'EMA-FC: gmean decrease >17; and OF-FC <22.8% residual red cells. All tests were performed within 24 hours from sampling. Criteria to confirm HS diagnosis was presence of spherocytes in the blood smear associated to positive results in at least two tests. **Results.** HS was diagnosed in 18 cases. Either CH or 5'EMA-FC were positive in 17 of them (94%), whereas OF-FC was positive in 16 of 16 HS children in whom the test was performed (100%). The only false positive result was CH in a newborn with hemolytic disease of the newborn secondary to ABO incompatibility. Most HS patients were positive for all performed tests. Only two of them presented one normal result, either for CH or for 5'EMA-FC tests. **Conclusions.** Neonatal jaundice is often the first manifestation of HS, associated to anemia in 50-80% of cases. Moreover, HS currently is a recognized cause of kernicterus. Therefore, HS is one main differential diagnosis to take into consideration when evaluating a neonate with hemolytic anemia. However, HS is usually underrecognized as a cause of neonatal hyperbilirubinemia. Our results show that the simultaneous use of these three tests allows confirming the diagnosis of HS in 100% of patients. Sensitivity was 94% for CH and 5'EMA-FC, and 100% for OF-FC, in agreement with those reported for the standard use of venous blood. The use of very small blood volumes allows an earlier etiological diagnosis in neonates and small infants. These results need to be confirmed in larger populations including HS and non-HS patients.

0398

DEVELOPMENT, IMPLEMENTATION AND FOLLOW-UP OF A NEW MANAGEMENT TOOL TO IMPROVE BLOOD TRANSFUSION SAFETY

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Background. Assuring transfusion safety is an essential element of health

care in all countries, requiring government commitment and a legal framework. Fundamental transfusion safety strategies include selection of low risk donors, Good Manufacturing Practices in preparation of blood components, and appropriate clinical use of blood products including avoidance of unnecessary transfusions. To assure the latter, most centers employ an educational approach focused on the prescribing physician, associated with pre-transfusional audits of transfusion adequacy. However, the implementation of these laborious measures is often insufficient to prevent the unnecessary use of blood components, so that measures to monitor the efficacy of these strategies are key to blood transfusion services. **Aims.** Here we evaluate the efficacy of the utilization of a simple and straightforward management tool developed fours years ago, and used ever since, to monitor the use of blood components and enhance blood transfusion safety, in a private transfusion service in Campinas, SP, Brazil **Methods.** In our Institution, all transfusion requests are audited by a transfusion medicine physician prior to transfusion, and classified as "proper"or "non-proper"based on internationally-adopted guidelines regarding transfusion indications. "Non-proper"requests are then adapted to suit these protocols. From April to October 2008, requests for red blood cells (RBC), were additionally analyzed in order to identify recurrent characteristics that could be associated with "non-proper"requests. Two characteristics were associated with a higher likelihood of "non-proper"indication: (1) pre-transfusion hemoglobin higher than 9.0 g/dL, and (2) absence of information on hemoglobin levels. These two markers were then considered as surrogate markers of adequate use of RBC. These two characteristics were then used to calculate the so-called "RBC transfusion adequacy index"(RAI), which represents the proportion of RBC transfused in which any of these two characteristics were present. RAI was monitored prospectively from november 2008 to january 2012, as an indicator of the efficacy of other measures implemented to promote adequate use of blood products. **Results.** During the first part of the study, 1495 transfusions requests were analyzed, of which12,9% were classified as "non-proper". In the second part of the study, from november 2008 until january 2012, the RAI was used as a surrogate monitor of adequate use of blood transfusion monthly and the mean value was 85,46 % ± 2,42(SD). Regardless of the knowledge that blood transfusions are associated with significant risks, inadequate use of blood is still a problem. Available tools to avoid unnecessary transfusions are hard to implement in day-to-day practice. The acceptable RAI can vary significantly from service to service according to hospital complexity, social-economic issues and resources in different institutions. Summary: The optimal management of blood transfusion and patient related risks are a on course task. Here we have, prospectively shown that, RAI can be helpfull in transfusion services to monitor the efficacy of adequate use of blood components.

0399

REDUCING THE RISK OF VCJD TRANSMISSION: A SAFETY TRIAL OF PRION-FILTERED VERSUS STANDARD RED CELLS TRANSFUSION IN SURGICAL PATIENTS (PRISM)

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Background. Variant Creutzfeldt-Jakob (vCJD) is a fatal disease which can rarely be transmitted via blood transfusion. No reliable screening test exists for blood donors, and in animal models leucodepletion of donated red cells does not completely remove prion infectivity. A red cell filter device P-Capt™ (Macopharma) incorporating a resin designed specifically to remove prions has been CE marked for use inEurope. **Aims.** The PRISM study was designed to examine: 1. possible serious clinical sequelae in patients following transfusion of red cells that have been P-Capt® filtered, namely an increase in the formation of new, clinically significant red cell antibodies and; 2. an increase in atypical or previously recognised transfusion reactions. All outcome variables were measures of safety. **Methods.** Adult surgical patients in 9UK hospitals who required transfusion support were entered into a multicentre, non-randomised, open, controlled trial. All patients gave informed consent. Most patients had complex or re-do cardiac or vascular surgery. Patients who received at least 1 unit of filtered red cells were compared with a control cohort of patients who received standard red cell units only. The primary outcome was the development of red cell antibodies at 8 weeks or 6 months post-transfusion. Secondary outcomes included transfusion reactions and atypical symptoms arising during or post-transfusion. Imputability of these to transfusion was allocated after central review. **Results.** 917 P-Capt® filtered and 1336 standard red cell concentrate (RCC) units were transfused into 590 patients. 291 patients received standard red blood cells only, and 299 patients received at least one unit of P-Capt® filtered red blood cells. 26 new red cell antibodies were detected in 20 patients (10 standard arm; 10 filtered arm) with an overall alloimmunisation rate of 4.4% for patients who provided at least one follow up blood sample (78% of those transfused). Analysis by exact logistic regression showed that neither the treatment arm nor the number of units transfused had a significant effect on the proportion of patients who developed new clinically significant antibodies (OR 0.93, 95% CI 0.3, 2.5) and (OR 0.95, 95% CI 0.8, 1.1) respectively. No pan-reactive antibodies or new red cell antibodies formed specifically against P-Capt® filtered red cells were detected.Only 1 of 26 reported transfusion-related events was considered a probable transfusion reaction (febrile reaction, standard arm). A further 17 were considered possibly or unlikely to be transfusion-related (6 standard arm, 11 filter arm, p = 0.369), and 8 further events, all in the filter arm, were considered to be unrelated to the transfusion. None of the SAEs reported in the study (134 standard arm; 114 filtered arm) was considered clinically unexpected for this population or directly related to transfusion. **Conclusions.** Transfusion of P-Capt® filtered cells does not appear to increase the rate of red cell alloimmunisation and there were no transfusion reactions or adverse events clearly related to the filter in this study. Our findings suggest that these filters do not reduce the overall safety of transfusion, but post-marketing surveillance would be needed if implemented.

0400

PREVALENCE AND PATTERN OF HEPATITIS B VIRAL MARKER DETECTION IN BLOOD DONORS AT CANADIAN BLOOD SERVICES HALIFAX/PEI

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Background. Current Canadian Blood Services (CBS) testing for the presence of the Hepatitis B virus (HBV) in blood donors involves universal screening for HBV DNA, Hepatitis B surface antigen (HBsAg) and Hepatitis B core antibody (anti-HBc). Supplementary testing includes Hepatitis B surface antibody (anti-HBs). **Methods.** A retrospective review of donor files testing positive for a HBV marker at CBS Halifax/PEI from January 1, 2011 to February 1, 2012 was undertaken to characterize the test result pattern. All donors denied prior hepatitis and vaccination within the past three months on their Record of Donation. Information about previous HBV vaccination was obtained from donors who contacted CBS about their HBV-positive test results. **Results.** Thirty-six donors tested negative for HBV DNA but reactive to one or more HBV seromarkers during the study period. Six donors tested anti-HBc repeat-reactive (RR) only. Twenty-seven donors tested RR for both anti-HBc and anti-HBs (17 new and 10 returning donors). Of the 10 returning donors who tested RR for anti-HBc and anti-HBs, 4 were documented to be HBsAg-negative < 3 months prior to the reactive donation. Another four out of the 27 RR anti-HBc and anti-HBs reactive donors were documented to have had HBV vaccination remotely. Three scenarios were observed for three donors who tested HBsAg-RR: HBsAg neutralization negative with reactive anti-HBs; HBsAg neutralization positive with non-reactive anti-HBs; and HBsAg neutralization negative with anti-HBs not tested. The donor testing RR for HBsAg (HBsAg neutralization positive) and anti-HBs non-reactive received a vaccination within days of donation. **Conclusions.** The majority of donors testing positive for a HBV seromarker were found to be RR for anti-HBc and reactive for anti-HBs which in conjunction with a non-reactive HBsAg and negative HBV DNA, most likely reflects previous, resolved acute HBV infection. An isolated RR anti-HBc result among 6 donors likely reflects either a false-reactive anti-HBc or a remote, resolved acute HBV infection with loss of anti-HBs. Recent HBV vaccination may cause a transient reactive HBsAg. Acquiring information from donors on the status of prior HBV vaccination may be of use in interpreting HBV test results.

0401

THE EFFICACY AND SAFETY OF CRYOPRECIPITATE FOR FIBRINOGEN REPLACEMENT IN ACUTE AND CHRONIC ACQUIRED HYPOFIBRINOGENAEMIA

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Background. Fibrinogen is essential for coagulation especially in the context of acute haemostatic challenge such as surgery or haemorrhage. Cryoprecipitate is a source of fibrinogen and established as a treatment for acquired hypofibrinogenemia. It is produced from pooling of plasma fractions from a number of donors. In recent years, virally-inactivated fibrinogen concentrates have become available and are now considered the treatment of choice for congenital hypofibrinogenemia based on greater predictability of response and

safety profile. Despite its common use there is limited data on the efficacy and safety of cryoprecipitate in patients with acute and chronic hypofibrinogenemia. **Aims.** This retrospective study examines the efficacy and safety of cryoprecipitate in raising plasma fibrinogen concentrations and improving coagulation and clinical parameters in the treatment of patients with acute and chronic acquired hypofibrinogenemia. **Methods.** A retrospective analysis of data collected for all patients receiving cryoprecipitate at a large teaching hospital in the UK between October 2009 and August 2010 was undertaken. Overall, 132 cryoprecipitate transfusion events occurred over the study period. Of these, 79 episodes met the inclusion criteria. Plasma fibrinogen levels, Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT) were recorded prior to cryoprecipitate administration and subsequent levels were noted at both 24 and 72 hour time-points. Medical records were consulted to determine the indication for cryoprecipitate administration, clinical response and any subsequent transfusion-related morbidity and mortality. The patients were followed up to a period of 1 year to detect any transfusion-acquired infections. Statistical analysis was carried out using non-parametric paired tests (Wilcoxon test). **Results.** On average 2 pools of cryoprecipitate - constituting a typical adult dose - were transfused at each event. Across all groups cryoprecipitate significantly raised the plasma fibrinogen level by a median of 0.8 g/L (P<0.001) though there was variation in the individual response of patients. Coagulation parameters also improved with significant reductions in both the PT (P<0.01) and aPTT (P<0.001). Correction in coagulation parameters correlated with improvement in clinical condition and cessation of bleeding in most cases. No increase in morbidity or mortality was observed attributable to cryoprecipitate transfusion. Additionally, after a year of follow up there were no reports of transfusion-related infections in any of the patients. Interestingly, the rise in plasma fibrinogen was particularly marked in patients who were bleeding acutely and had no evidence of a pre-existing coagulopathy. Patients with chronic hypofibrinogenemia had a significantly lower and less sustained response to cryoprecipitate. **Conclusions.** Cryoprecipitate remains an effective method of increasing the plasma fibrinogen level and subsequently improving the clotting of patients who are bleeding acutely with a low fibrinogen level. It is most effective in patients with no chronic underlying cause for their hypofibrinogenemia. However, the fibrinogen content of a pool of cryoprecipitate can vary significantly and clinical response may therefore be less reliable than with the use of purified fibrinogen concentrates. In addition, concerns regarding the theoretical risk of transfusion-related infection with the use of pooled cryoprecipitate remain, especially in the context of virally-inactivated concentrate now being readily available at a comparable cost.

Table 1. Fibrinogen response to cryoprecipitate transfusion in acute and chronic hypofibrinogenemia.

		Fibrinogen Level (g/L)					
Indication	**N (%)**	**Baseline**		**24 hour**		**72 hour**	
Acute Fibrinogen Deficiency	*41 (52)*	***0.90***	*(0.5)*	***2.00****	*(1.6)*	***2.90****	*(2.5)*
Bleeding	15 (19)	**0.90**	*(0.4)*	**2.50**	*(1.1)*	**3.60**	*(1.5)*
DIC	11 (14)	**0.60**	*(0.4)*	**1.10**	*(0.8)*	**1.70**	*(0.7)*
Liver Transplantation	8 (10)	**0.80**	*(0.3)*	**2.00**	*(0.9)*	**3.90**	*(1.4)*
Surgery	7 (9)	**1.40**	*(1.1)*	**2.45**	*(1.7)*	**3.50**	*(1.3)*
Chronic Fibrinogen Deficiency	*38 (48)*	***1.00***	*(0.5)*	***1.50****	*(0.8)*	***1.30***	*(0.5)*
Hepatic Insufficiency	27 (34)	**1.00**	*(0.5)*	**1.50**	*(0.8)*	**1.30**	*(0.5)*
Haematological Malignancy	10 (13)	**1.15**	*(0.6)*	**1.45**	*(0.4)*	**1.30**	*(0.6)*
Chronic Malnutrition	1 (1)	**1.40**	-	**2.60**	-	**2.50**	-
Total	*79*	**0.90**	*(0.7)*	**1.70**	*(0.9)*	**1.70**	*(1.5)*

Values are **Median** *(Interquartile Range)*

**<0.01 compared to baseline value

***p<0.001 compared to baseline value

0402

STUDY OF EFFICACY RED BLOOD CELL TRANSFUSIONS AND THEIR INFLUENCING ON QUALITY OF LIFE IN HEMATOLOGICAL MALIGNANCIES PATIENTS WITH ANEMIA

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Background. Anemia in hematological malignancies (HM) patients is a frequent symptom, decreased survival rate and overall quality of life (QoL). Red blood cell (RBC) transfusions are routinely used to correct severe anemia increasing hemoglobin (Hb), improving patient's status and QoL in a short time. **Aims.** 1. To study the efficacy RBC transfusions. 2. To investigate QoL before and after RBC transfusions in anemic patients. **Methods.** HM patients (n=61) with anemia: non-Hodgkin's lymphoma (n=5), chronic lymphocytic leukemia (n=12), multiple myeloma (n=14), primary myelofibrosis (n=8), myelodysplastic syndrome (n=11), acute myeloid (n=9) and lymphoid leukemia (n=2). The median age of patients was 64.0 years (range 21-82). Transfusion threshold was Hb concentration <8.0 g/dL (for young patients; n=50) and <9.5 g/dL (for elderly patients; n=11). The Hb level was studied before and after every transfusion. QoL was assessed using FACT-An questionnaire: all patients filled up the special forms of questionnaire before the first RBC transfusion and after the latest one. Most patients were receiving chemotherapy according their diagnoses. The target Hb level was >8.0-9.9 g/dl. **Results.** Mean baseline Hb concentration was 7.0±1.2 g/dL (4.0-9.2 g/dl). A number of transfusions was 1-9 (median 3) during the hospitalization period (4-35 days). The Hb concentration significantly increased from baseline to 9.3±0.9 g/dl (8.0-10.2 g/dl; p<0.01). In whole group of patients Hb concentration increased at 2.8±1.3 g/dL after all RBC transfusions. Every blood unit increased Hb level at 0.9±0.3 g/dL on average. Only 28 patients (45.9%) increased Hb ≥1.0 g/dL after every transfusion, but 33 (54.1%) - 0.1-0.9 g/dL. Out of them were five chemotherapy refractory patients (8.2%), who increased Hb only at 0.1-0.4 g/dL per unit, and twenty eight ones (45.9%) who increased Hb at 0.5-0.9 g/dL, they were patients after high dose chemotherapy (n=6), progression of disease and refractory to chemotherapy (n=18), and severe hemolysis (n=4). FACT-An demonstrated that RBC transfusion reduced symptoms (items) of anemia in most cases (Figure): 1) I feel fatigued; 2) I feel weak all over; 3) I feel listless ("washed out"); 4) I feel tired; 5) I have trouble starting things because I am tired; 6) I have trouble finishing things because I am tired; 7) I have energy; 8) I have trouble walking; 9) I am able to my usual activities; 10) I need sleep during the day; 11) I feel lightheaded; 12) I get headaches; 13) I have been short of breath; 14) I have pain in my chest 15) I am too tired to eat; 16) I am interested in sex; 17) I am motivated to do my usual activities; 18) I need help doing my usual activities; 19) I am frustrated by being too tired to do the things I want to do; 20) I have to limit my social activity because I am tired. But statistically significance (p<0.05) were observed in 1-6 and 11-13 items (points reduced from 2.50 to 1.41 on the average). **Conclusions.** The study has shown that RBC transfusions not only are effective increase Hb level in anemic HM patients but also improve QoL.

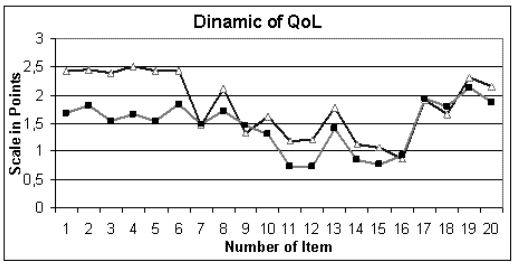

Figure 1.

0403

STUDY QUALITY OF LIFE IN LYMPHOPROLIFERATIVE DISORDERS PATIENTS WITH ANEMIA TREATED ERYTHROPOIETIN

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Background. Anemia in lymphoproliferative disorders (LPD) patients is a frequent symptom and can decrease the efficacy of antitumor chemotherapy, survival rate and overall quality of life (QoL). Its pathogenesis is based on suppression by proinflammatory cytokines and decreasing erythroid precursor's sensitivity to serum erythropoietin. Therefore recombinant human erythropoietin (rHuEPO) are used as a pathogenetic therapy of anemia in patients with LPD which significantly increase hemoglobin, reduce a number of RBC transfusions and improve QoL. **Aims**.To study the efficacy of rHuEPO and improvment QoL in LPD patients with anemia. **Methods.** There were done this interventional prospective study to investigate the efficacy of rHuEPO in reducing RBC transfusion-dependency, increasing Hb concentration and QoL in patients (n=103) with low-grade non-Hodgkin's lymphoma (n=18), chronic lymphocytic leukemia (n=23) and multiple myeloma (n=62). The median age of patients was 65.5 years (range 24-84). rHuEPO was injected subcutaneously on 450 IU/kg weekly. Before start of rHuEPO treatment all patients have being received two or

more cycles of antitumor chemotherapy. The patients with Hb concentration <8.0 g/dL received RBC transfusions before rHuEPO treatment. The target Hb level was 12 g/dL. The duration of treatment with rHuEPO was planned within 16 weeks. Positive response was estimated as increasing Hb concentrating ≥2.0 g/dL or achieving target Hb level (12 g/dl) during the period of rHuEPO therapy and so achieving RBC transfusion-independency. QoL was assessed using the FACT-An questionnaire. **Results**. Mean baseline Hb concentration was 8.71±1.46 g/dl (3.7-10.0 g/dl). Before rHuEPO-therapy 31 patients had received RBC transfusion (1-17 units) during last 3-6 months because of low Hb (3.7-8.0 g/dl). The period of rHuEPO-therapy was from 4 to 16 weeks (mean 9.5±3.7 weeks). During the study period 14 patients (45.2%) followed RBC transfusions after rHuEPO treatment and 17 patients (54.8%) showed transfusion-independency. Whole we observed positive response in 67 patients (65.0%), their Hb concentration increased from baseline to 12.5±1.0 g/dl (11.2-15.7 g/dL; p<0.001). FACT-An demonstrated that rHuEPO-therapy significantly (p<0.02) reduced a range of anemic symptoms (Items 1-6 and 13; Figure): 1) I feel fatigued; 2) I feel weak all over; 3) I feel listless ("washed out"); 4) I feel tired; 5) I have trouble starting things because I am tired; 6) I have trouble finishing things because I am tired; 7) I have energy; 8) I have trouble walking; 9) I am able to my usual activities; 10) I need sleep during the day; 11) I feel lightheaded; 12) I get headaches; 13) I have been short of breath; 14) I have pain in my chest 15) I am too tired to eat; 16) I am interested in sex; 17) I am motivated to do my usual activities; 18) I need help doing my usual activities; 19) I am frustrated by being too tired to do the things I want to do; 20) I have to limit my social activity because I am tired. **Conclusions.** The study has shown that rHuEPO is effective treatment of reducing RBC transfusion-dependency, increasing Hb and improving QoL in LPD patients with anemia.

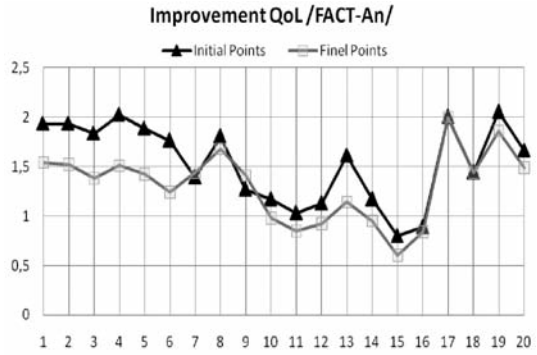

Figure 1.

0404

PHARMACOECONOMIC BURDEN FOR DELIVERY OF ANTI-RETROVIRAL TREATMENT: WHAT ARE WE IN FOR?

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Background. Although the safety of transfused blood products has been continuously improving, even today, we still see transfusion transmitted viral infections. **Objective**: Our objective was to assess the pharmacoeconomic impact of antiretroviral therapy and highlight the occurrence of transfusion transmitted diseases [TTD]. **Methods.** Retrospective analysis of our Hemophilia A database. HIV1 and 2 were tested by ELISA followed by further testing with western Blot in retrovirus positive samples. Retrovirus viral load was monitored by quantitative PCR, and CD4 counts before and during treatment. CD4 counts were monitored by flow cytometry. **Results.** Amongst 93 Hemophilia A patient in our database since 1992, 17 (18.3%) had TTD. 13 (76.5%) patients had Anti-HCV positivity [6 HCV RNA PCR positive]; 2 (11.75%) were HIV-1 positive by western blot [both were retrovirus RNA PCR positive]; and 2 (11.75%) were HBV positive [both were also HBV DNA positive]. Two of these patients were positive for all the three viruses together. The two retrovirus RNA PCR positive cases, achieved a sustained virological remission [SVR] on the currently recommended HAART regimen following treatment over 27 and 32 months respectively. Furthermore, the CD4 counts have also normalized following SVR. Interestingly, we also observed an increased tendency for bleeding, especially when treatment was intensified by using boosted darunavir in one patient, which was controlled with prophylactic Factor VIII:C transfusions. The current cost of treatment [US dollars] for these two patients has been 16,120 and 15,172 over the past 12 months of treatment, respectively. Furthermore, the cost [US dollars] of prophylaxis with Factor VIII:C [15-25 units/kg, twice a week is 41,967 (annual) for the 2 patients. **Summary and Conclusions.** We report here SVR in two retroviral infections with RNA PCR negativity in both the treated patients. However, anti-retroviral treatment is ongoing according to the currently recommended international guidelines along with Factor VIII:C prophylaxis. Thus, the pharmacoeconomic burden with the average current cost of treatment for antiretroviral therapy and Factor VIII:C prophylaxis is about 36,630 USD, per patient in the long term.

0405

EVALUATION OF A SINGLE 1000 MG IRON DOSE FERRIC CARBOXYMALTOSE FOR IMPROVEMENT OF FATIGUE IN IRON DEFICIENT, NON-ANEMIC, PREMENOPAUSAL WOMEN IN THE RANDOMIZED, PLACEBO-CONTROLLED STUDY PREFER

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Background. In iron-deficient, non-anemic (IDNA) women, repeated iron administration can reduce symptoms of fatigue. **Aims.** This study evaluated the efficacy and safety of a single 1000 mg iron dose of intravenous (i.v.) ferric carboxymaltose (FCM) in healthy premenopausal women suffering from symptomatic unexplained fatigue and iron deficiency. **Methods.** Eligible women had to be iron deficient (serum ferritin <50 ng/mL and transferrin saturation TSAT <20%, or serum ferritin <15 ng/mL) but non-anemic (hemoglobin ≥11.5 g/dL) and present with a total score ≥5 on the Piper Fatigue Scale (PFS). Patients with major depressive disorder, any active or unstable concurrent medical condition, known infections, chronic inflammation, use of iron preparations within 4 weeks prior screening or a body weight <50 or >90 kg were not included. Participants were blinded to the study drug, randomized to receive a single dose of FCM (1000 mg iron) or saline solution (placebo), and followed-up after 7, 28, and 56 days. **Results.** 290 of 294 treated women were included in the analysis (FCM 144, placebo 146). Baseline characteristics were similar in both groups. Total PFS score improved (decreased by ≥1 point from baseline to Day 56) in 65.3% (FCM) and 52.7% (placebo) of patients (primary endpoint, odds ratio 1.68, 95%CI 1.05-2.70; p=0.03). The calculated improvement in total PFS was statistically significantly higher in the FCM (2.2±2.1 points) vs. the placebo group (1.4±2.0 points) (mean±SD; p=0.0007) and also consistent for all subscores on behavioral (2.46±2.31 vs. 1.56±2.26; p=0.001), affective (2.52±2.33 vs. 1.55±2.26; p<0.001), sensory (2.14±2.34 vs. 1.38±2.26; p=0.003) and cognitive mood (1.70±2.06 vs.0.98±1.94; p=0.003) aspects. Also computerized visual analogue scale (VAS) scores of self-rated alertness (14.2±18.0 vs. 8.9±17.9; p=0.007) and contentment (9.3±16.8 vs. 4.6±16.16; p=0.023) improved better after FCM compared to placebo treatment; differences in self-rated calmness showed a strong trend in favor of FCM (10.0±20.5 vs. 5.0±18.2; p=0.077). Correspondingly, the SF-12 mental score improved by 5.8±9.8 vs. 2.8±8.9 points (p=0.006). Notably, significant improvements in the different scores were associated with baseline TSAT <20%. At Day 56 after FCM treatment, serum ferritin levels were normal (≥50 ng/mL) in 99.3% of FCM-treated patients (placebo 2.1%), and 76.4% (placebo 32.9%) had a normal TSAT of 20-50%. Treatment emergent adverse events (114 with placebo and 209 with FCM) were mainly mild in intensity (63.2% and 66.5%, respectively). **Conclusions.** A single dose of FCM (1000 mg iron) effectively reduced fatigue symptoms and improved patient-rated mental quality of life as well as cognitive function in iron-deficient but otherwise healthy and non-anemic women. The results of this placebo-controlled study confirm that iron deficiency even without anemia, particularly if TSAT is below 20%, can affect women's health, and emphasize the importance of maintaining a normal iron status independent of Hb levels.

Hemoglobinopathy 1

0406

EFFECT OF COMBINED ANTIOXIDANT THERAPY ON LIVER IRON CONCENTRATIONS AND HEPATIC FIBROSIS IN BETA-THALASSEMIA MAJOR PATIENTS

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Background. Repeated blood transfusions lead to oxidative tissue injury in β-thalassemia major (β-TM) due to iron overload which may enhance liver injury and fibrosis. **Aims.** to assess the effects of combined vitamin therapy on oxidant-antioxidant capacity, hepatic status and hemoglobin derivatives in β-TM patients. **Methods.** Prospective follow up study for 12 months involved 60 β-TM patients aged 4-17 years compliant on deferiprone (> 85% of calculated dose) compared to 60 healthy controls. Patients with initial low serum vitamins E, C, A (group1) were treated with therapeutic oral vitamin doses. Investigations done included: vitamins (A,C,E) levels, liver transaminases, serum ferrittin, percentage of erythrocyte hemolysis, hepatic fibroscan elastography (TE) for liver fibrosis and hepatic MRI R2* for liver iron concentration (LIC) were performed before, after 6 and 12 months of therapy. Antioxidant capacity assessed using reduced glutathione, malondialdehyde (MDA), catalase, superoxide dismutase and glutathione enzymes. **Results.** In β-TM group1 (n=39); baseline vitamin A, E, C, hemoglobin and reduced glutathione levels were significantly lower while MDA was significantly higher compared to controls (p<0.001). Correlation studies revealed that basal MDA level in β-TM was positively correlated with basal LIC (r=0.44,p<0.001) and ALT(r=0.52,p<0.001) while it was negatively correlated with basal vitamin A(r=-0.74,p<0.001), vitamin C(r=-0.65,p<0.001), vitamin E levels(r=-0.63,p=0.001) and glutathione peroxidase (r=-0.32, p=0.04). Initial serum ferritin level was negatively correlated with basal levels of vitamin A (r=-0.28, p=0.04) and vitamin E(r=-0.36,p=0.04). Basal LIC values were negatively correlated with vitamin A(r=-0.38,P=0.002), vitamin E(r=-0.45, p<0.0001) and vitamin C(r=-0.27, p=0.0), while it was positively correlated with basal ALT(r=0.43,p=0.001). Stepwise regression analysis was done and revealed that the most significant effect on MDA level in studied thalassemics were the initial values of LIC, vitamin A followed by AST levels. Follow up of sttudied thalassemics revealed that; vitamins, reduced glutathione and hemoglobin levels were significantly elevated and paralleled by progressive decline in MDA, reticulocytic count and serum ferritin levels during antioxidant therapy (p<0.001). Liver transaminases, superoxide dismutase levels were significantly decreased while glutathione reductase was significantly elevated during antioxidant therapy (p<0.001). Minimal Improvement of hepatic fibrosis as 23% of patients had TE (>12kPa) at baseline compared to 20.5% after 12 months, while 48.7% initially had TE (>6kPa) compared to 51.2%, 56.4% at 6 and 12 months. While LIC values were significantly decreased (mean; 16.4, 11.3, 8.9 mg/gm at baseline, after 6, 12 months, p<0.001) after antioxidant therapy. **Conclusions.** increased oxidative damage in β-TM may be due to depletion of lipid soluble antioxidant vitamins. Combined vitamins; A,C and E therapy improves the antioxidant status, reduce hemolysis rate and improve the hepatic status both by decrease LIC and to less extent the degree of liver fibrosis in β-TM compliant on deferiprone.

0407

CIRCULATING PLATELET AND ERYTHROCYTE MICROPARTICLES IN SICKLE CELL DISEASE: RELATION TO CLINICOPATHOLOGICAL CHARACTERISTICS AND VASCULAR COMPLICATIONS

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Background. Sickle cell disease (SCD) is characterized by a complex vasculopathy, consisting of endothelial dysfunction and increased arterial stiffness, with a global effect on cardiovascular function. The hypercoagulable state and thromboembolic complications in those patients may result from multiple implicated factors, including chronic hemolysis and circulating cell-derived microparticles which originate from activated platelets and erythrocytes. **Aims.** This study aimed to measure the levels of platelet and erythrocyte-derived microparticles (PMPs and ErMPs) in young children and adolescents with sickle cell disease ,and their relation to the clinicopathological characteristics and aortic elastic properties. **Methods.** The study is a cross sectional study ,included 40 patients with sickle cell disease (aged 5-17 years) regularly followed in the Pediatric Hematology Unit,Ain Shams University , in Cairo,Egypt ,compared to 40 age and sex matched healthy controls. Patients were studied stressing on frequency and severity of sickling crisis, transfusion therapy, hydroxyurea and chelation therapy, as well as hematological profile, hemoglobin electrophoresis, D-dimer assay and antithrombin III (AT III) .Circulating platelet microparticles PMPs ($CD41b^+$) and erythrocyte-derived microparticles ErMPs (glycophorin A^+) were determined by flow cytometry. Echocardiography was performed with assessment of aortic elastic properties: aortic stiffness and distensibility. **Results.** Both PMPs and ErMPs were significantly increased in SCD patients compared to healthy controls (p<0.001). The highest MPs levels were found in patients with sickle cell crisis compared with those in steady state (p<0.001). SCD patients on hydroxyurea therapy showed lower values compared with untreated patients (p<0.001). Both types of MPs were inversely correlated with hemoglobin level (p=0.01) and positively correlated with lactate dehydrogenase (p=0.001), indirect bilirubin (p= 0.001), and D-dimer levels (p<0.05).Only ErMPs levels were positively correlated with serum ferritin (p=0.02). Echocardigraphic findings showed that systolic left ventricular function was preserved in all studied patients, while 14% of SCD patients had diastolic dysfunction. Aortic distensibility was significantly decreased while aortic stiffness was significantly increased in SCD compared to controls (p<0.05,for both). Aortic distensibility was negatively correlated with transfusion index in SCD patients (P=0.001). Aortic stiffness index was positively correlated to disease duration,PMPs, ErMPs, LDH and serum ferritin levels in studied patients (p<0.05). Aortic stiffness was positively correlated with intial Hb S% at time of diagnosis and with severity and numbers of sickling crisis in SCD patients(P=0.01, p=0.02 respectively). **Conclusions.** We suggest that increased circulating PMPs and ErMPs may be considered potential biological markers for vascular dysfunction and disease severity in SCD and may be implicated in the pathogenesis of coagulation abnormalities encountered in these patients. Their levels are closely related to markers of hemolysis, sickling severity, fibrinolysis and iron overload.

0408

SOLUBLE CD163 AS A BIOMARKER OF DISEASE SEVERITY IN YOUNG SICKLE CELL DISEASE PATIENTS AND THEIR TRAIT SIBLINGS

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Background. CD163 is a member of the Scavenger-receptor Cysteine-rich family of proteins. It is expressed on cells of monocyte-macrophage lineage and is the main hemoglobin-haptoglobin receptor ,and mediates the interaction between macrophages and erythroblasts . In SCD ,inflammation and monocyte activation are predisposing factors to vaso-occlusion crises. Siblings of patients with SCD may have the same pathophysiology without displaying severe symptoms of SCD. Soluble CD163 (sCD163) is a valuable biomarker in diseases with monocyte-macrophage involvement. Its clinical relevance in sickle cell disease (SCD) and trait remains elusive. **Aims.** This study aimed to assess (sCD163) levels in children with SCD and their sickle cell trait (SCT) siblings in relation to clinicopathological characteristics and to investigate its value as a potential marker for disease severity and hydroxyurea treatment response. **Methods.** 60 SCD patients, 30 SCT siblings and 30 healthy controls (median age : 9 years,7.3 years,and 7.5 years, respectively) were included. SCD patients were studied in the steady state excluding patients who had sickling crisis or other inflammatory conditions within one month prior to the study . All were subjected to history taking stressing on frequency of sickling crisis, transfusion requirements and response to hydroxyurea therapy. Investigations included hematological profile, hemoglobin electrophoresis,serum ferritin and measurement of serum sCD163 levels(ELISA). One year clinical and laboratory follow-up was done for studied patients and siblings. **Results.** Serum sCD163 levels were elevated in SCD patients and SCT siblings compared to controls (p<0.001), levels in untreated SCD were higher than in SCT siblings (p<0.001). sCD163 levels were higher in SCD patients who developed sickling crisis during one year study period (p<0.05). Patients on hydroxyurea therapy had lower sCD163 compared to untreated patients (p<0.001). There was significant positive linear relationships between sCD163 and age, disease duration, total leucocytic count and HbS with negative correlations with mean hemoglobin and HbF levels in SCD (p<0.05) . In siblings, sCD163 was positively correlated with age, HbS and negatively correlated with hemoglobin and HbF (p<0.05). Stepwise regression analysis showed that total leucocytic count, HbS and HbF were independently related to sCD163 in SCD (r2 =0.968, p<0.001). ROC curve analysis showed that the cutoff value of sCD163 at 1400 ng/mL could be considered a predictor for sickling crisis differentiating patients with and without the occurrence of this complication with a sensitivity of 92.3% and specificity of 94.1% (AUC, 0.98; 95% CI, 0.95-1.01; p<0.001) . **Conclusions.** sCD163 levels are related to SCD patients' clinical condition and can be considered a prognostic biomarker for early crisis prediction and to monitor response to hydroxyurea therapy .Elevated sCD163 in trait siblings could reflect increased risk of sickling in challenging situations.

0409

FLOW CYTOMETRY ASSESSMENT OF CIRCULATING PLATELET AND ERYTHROCYTE MICROPARTICLES IN THALASSEMIA MAJOR PATIENTS: RELATION TO CLINICAL, LABORATORY AND ECHOCARDIOGRAPHIC FINDINGS

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Background. Thalassemia is a hereditary hemolytic anemia caused by mutations in the globin gene complex. Circulatory disturbances including arterial and venous thrombosis have been reported. Aggregability of abnormal red cells and the high level of membrane-derived microparticles stemming from activated platelets and other blood cells are thought to be responsible for the associated thrombotic risk. Hemolysis is also thought to be an important pathophysiological prothrombotic risk, particularly through the formation of circulating vesicles. **Aims.** This study aimed to measure the levels of platelet and erythrocyte-derived microparticles (PMPs and ErMPs) in children and adolescents with β-thalassemia major (TM), and their relation to the clinical and laboratory parameters as well as aortic elastic properties and echocardiograpic findings. Methods:The study is a cross sectional study ,included 40 patients with β-thalassemia major (TM) (aged 8-19 years) regularly followed in the Pediatric Hematology Unit, Ain Shams University, in Cairo, Egypt ,compared to 40 age and sex matched healthy controls. Patients were studied stressing on transfusion and chelation therapy,splenectomy, history of thrombotic events, as well as hematological profile, hemoglobin electrophoresis, D-dimer, antithrombin III (AT III). Circulating platelet microparticles PMPs ($CD41b^+$) and erythrocyte-derived microparticles ErMPs (glycophorin A^+) were determined by flow cytometry. Echocardiography was performed with assessment of aortic elastic properties: aortic stiffness and distensibility . **Results.** Both PMPs and ErMPs were significantly increased in TM patients compared to healthy controls ($p<0.01$). Splenectomized TM patients had higher levels of PMPs and ErMPs thannonsplenectomized patients with significant positive correlation of PMPs levels to the duration since splenectomy ($p=0.04$). Levels of both types of MPs were inversely correlated with hemoglobin level and positively correlated with markers of hemolysis (lactate dehydrogenase and indirect bilirubin), and D-dimer levels in TM patients ($p<0.05$, for all). ErMPs levels were positively correlated with serum ferritin in TM patients($p=0.02$). History of thrombotic events was associated with significantly higher levels of both PMPs and ErMPs ($p<0.001$).Patients non-compliant to chelation therapy(receiving ≤ 60% of the chelating therapy dose) and patients with serum ferritin > 2500 ng/ml had significantly higher levels of PMPs and ErMPs compared to compliant patients and patients with serum ferritin less than 2500 ng/mL ($p<0.001$, for both). Echocardiographic findings showed that systolic left ventricular function was affected in 16% of studied patients, while 22% of patients had diastolic dysfunction. Aortic distensibility was significantly decreased while aortic stiffness was significantly increased in thalassemia patients compared to controls ($p<0.05$). Aortic distensibility was significantly lower in splenectomized thalassemic patients compared to non-splenectomized($P=0.001$). Aortic stiffness index was positively correlated to disease duration, PMPs, ErMPs, LDH and ferritin levels in studied thalassemics ($p<0.05$). **Conclusions.** We suggest that increased PMPs and ErMPs may be considered as potential biological markers for vascular dysfunction and hypercoagulable state in thalassemia patients increasing their risk of thrombotic events.

0410

SERUM ANGIOGENIN LEVEL IN PATIENTS WITH BETA THALASSEMIA AND SICKLE CELL DISEASE

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Background. The role of angiogenin as a marker of angiogenesis has not been investigated in thalassemia or sickle cell disease patients. **Aims.** To estimate the serum angiogenin level in children and adolescents with beta thalassemia and sickle cell disease, and its relation to possible risk factors related to increased angiogenesis in those patients. **Methods.** This study was conducted at the pediatric Hematology Clinic, Children's Hospital, Ain Shams University on 32 β-Thalassemia major (β-TM) patients(mean age 14.2±3.8 years; 18 splenectomized), 20 β-thalassemia intermedia (β-TI) patients (mean age of 14.3±4.8 years; two splenectomized) and 20 sickle cell disease(SCD) patients (mean age of 14.1±2.4 years; 4 splenectomized).SCD patients were subdivided into 8 with (HbSS) and 12 with sickle thalassemia (HbS/β-thalassemia). Thirty five age and sex matched healthy individuals served as controls. After taking informed consent, patients were subjected to clinical assessment, complete blood count, Hb electrophoresis, serum ferritin and serum angiogenin level was done by ELISA. **Results.** The angiogenin level was significantly higher in patients with SCD [250(100-300)pg/ml] compared to β-Thalassemia group [180(140-230)pg/ml] and controls [89(80-103) pg/ml] ($p<0.001$) especially those with HbSS disease ($p=0.06$). We found no significant difference in angiogenin level between β- TM and β-TI ($p=0.88$) or splenectomized and non splenectomized patients ($p=0.19$). There was a significant inverse correlation between angiogenin level and the age of patients in β-TM($p<0.001$) and β-TI ($P=0.009$) and significant negative correlation between angiogenin level and age of disease onset in β- TM ($r=-0.414$, $p=0.019$) and in β- TI ($r=-0.486$, $p=0.30$) but not in SCD. There was a significant inverse correlation between angiogenin level and ferritin in β- TI group($r=-0.573$, $p=0.008$). Moreover; a highly significant inverse correlation between serum angiogenin and total duration of chelation was detected in β- TI ($p<0.001$) and β- TM ($p=0.003$) groups. In SCD, there was a significant inverse correlation between angiogenin level and both frequency of blood transfusion ($r=-0.731$, $p<0.001$) and hydroxyurea duration of therapy ($p=0.017$). **Conclusions.** A significantly higher angiogenin level was detected among patients with SCD especially in severe form of SCD. Regular blood transfusion and hydroxyurea therapy may negatively influence angiogenin level in patients with SCD, while early starting age of blood transfusion and chelation therapy may decrease angiogenin level in patients with β-thalassemia major.

0411

RETROSPECTIVE ANALYSIS OF MUSCULOSKELETAL COMPLICATIONS IN ADULTS WITH SICKLE CELL DISEASE ATTENDING A SINGLE INSTITUTION OVER 21 YEARS

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Background. Musculoskeletal complications are common in patients with sickle cell disease (SCD). There is also a perception that the prevalence of connective tissue disease (CTD) in increased compared to populations without SCD. It is uncertain to what extent the presence of CTD affects the severity of SCD in these patients. **Aims.** This study assesses the prevalence of musculoskeletal complications including CTDs in the sickle cell adult cohort attending a teaching London hospital. In addition, it evaluates whether the presence of CTD correlates with increased severity of clinical course in patients with SCD. **Methods.** A retrospective investigational study was undertaken to assess musculoskeletal disease prevalence using serological and clinical documented evidence over a 21-year period, from 1999 - 2010. For the latter two years of our study period, patients with HbSS or HbSB0 (considered as sickle cell anemia, SCA) and CTD were compared against two markers of disease severity: a) number of hospital admissions and b) length of hospital stay. **Results.** 722 patients with SCD were included in the study group with 411 (57%) being women and 311 (43%) male. Genotype distribution shows hemoglobin S/S (HbSS) (n = 451), HbSC (n = 225), HbSB+ (n = 33) and HbSB0 (n = 13). 135 patients (18.7%) had ≥ 1 musculoskeletal disease(s) amongst the SCD population within the study period. Avascular necrosis (AVN) was the most prevalent musculoskeletal disease affecting 10% (n = 72), of the SCD population. Documented evidence of CTD was present in 3.1% (n = 23) of patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) being the most common at 2% (n = 14) and 0.3% (n = 2), respectively. Antinuclear antibody (ANA +ve) was found in 15% (n = 108) of patients and anti-smooth muscle antibody (ASM) present in 8% (n = 60) of patients. Within the latter two year period of the study, SCA patients with any positive autoimmune serology and/or CTD (n = 102) had a mean of 1.9 (±SD 2.7) admissions compared to a mean of 1.5 (±SD 2.7) admissions in those without autoimmunity and/or CTD (n = 210). The mean length of hospital stay in admitted patients was 6 days (±SD 4.1) in those with autoimmunity and/or CTD (n= 46) compared to 7 days (±SD 11.4) in those without (n = 108). **Conclusions.** Almost 1 in 5 patients in the SCD adult cohort had evidence of musculoskeletal disease. CTD prevalence of RA and SLE were greater in the SCD population in relation to prevalence reported in the existing literature in populations of similar ethnicity. There were no statistically significant differences in number of admissions or hospital stay between SCD patients with autoimmunity and/or CTD compared to those without. A considerable proportion of SCD patients were ANA +ve thus emphasizing the need for diagnostic vigilance in determining CTD in the patient with SCD.

0412

DIRECT AND INDIRECT INVOLVEMENT OF HCV INFECTION IN THE PATHOGENESIS OF MYOCARDIAL FIBROSIS IN THALASSEMIA MAJOR PATIENTS

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Background. In thalassemia major (TM), myocardial fibrosis has been detected using the late gadolinium enanched (LGE) cardiovascular magnetic resonance (CMR) technique and a positive correlation with anti-HCV antibodies has been described. However, HCV-induced cardiomyopathy is still controversial. **Aims.** The aim of our study was to verify a possible correlation between myocardial fibrosis detected by LGE CMR and chronic hepatitis C in a large retrospective cohort of TM patients. **Methods.** We analyzed 434 TM patients (233 males, mean age 31±9 years) consecutively enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) study. LGE images were acquired to detect myocardial fibrosis. HCV-RNA tests were sensitive to detect more than 50 copies/mlL **Results.** Ninety out of 434 TM patients (21%) were found to have myocardial fibrosis by LGE CMR technique. Among the 312 patients tested for HCV RNA there was a significant correlation between the presence of myocardial fibrosis and a chronic hepatitis C (P=0.011). Among the 62 patients with myocardial fibrosis tested for HCV RNA, we found a significant higher prevalence of diabetes mellitus in CHC patients versus the no CHC patients (P = 0.049). In all patients DM was developed after the HCV infection diagnosis **Conclusions.** Our finding supports the hypothesis that in the multi-transfused TM patients HCV infection can be involved in the pathogenesis of myocardial fibrosis through both myocarditis directly and the pancreas and liver damage with the development of diabetes indirectly. These patients could therefore benefit from therapeutic interventions directed towards the eradication of virus.

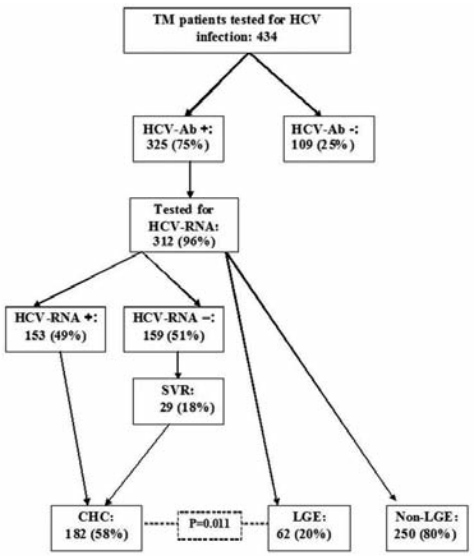

Figure 1. Flow chart of the test results.

0413

MATERNAL COMPLICATIONS AND THE ASSOCIATION TO BASELINE VARIABLES IN PREGNANT PATIENTS WITH SICKLE CELL DISEASE

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Background. Sickle cell disease (SCD) is an inherited hemoglobinopathy with multisystem complications. In pregnancy, it has been associated with multiple maternal complications. Studies addressing this issue have been limited in number and sample size. We planned to estimate the incidence of different maternal complications and the impact of baseline variables on the incidence. **Methods.** We retrospectively reviewed 68 consecutive pregnant patients with SCD followed at the obstetric clinic in a tertiary center from June 2006 to August 2011. We collected different baseline variables including age, gravidity, parity, baseline hematological parameters, history of splenectomy and cholecystectomy. We gathered the information on the number of SCD related admissions, emergency visits, intensive care admissions, acute chest syndrome, blood transfusions, pregnancy induced hypertension, preterm labor, maternal morbidity (eclampsia, uterine rupture and or post-partum hemorrhage), infection and maternal mortality. We used multivariable logistic regression to estimate and adjust the impact of baseline variables (age, parity, baseline hemoglobin F, history of splenectomy, cesarean section and the SCD genotype) on major maternal complications (define as a composite outcome of maternal mortality, morbidity, infection and pregnancy induced hypertension). **Results.** We analyzed 68 patients. The mean age was 30 years (standard deviation [SD] 3.8, range 22-40). The median gravidity and parity were 2 (range 1-6) and 1 (range 0-5) respectively. Sixty-two patients had SS genotype. The initial mean hemoglobin was 9.5 g/dl (SD 1.1, range 7.2-11.9). The mean baseline hemoglobin F was 10.2% (SD 6.6, range 0.7-29). All patients were on penicillin prophylaxis and folic acid. Five and eight patients had history of splenectomy and cholecystectomy respectively. Twelve patients delivered by cesarean section and only 1 patient had preterm labor. During pregnancy, 65 patients required admission for SCD and or pregnancy related complications (96%, confidence interval [CI] 91-100). The median number of admissions was 3 (range 0-20). Fifty-four patients (79%, CI 70-89) had at least 1 visit to emergency room with a median of 3.5 (range 0-75). Eight patients needed admission to intensive care unit (12%, CI 4-20). Fourteen patients developed acute chest syndrome (21%, CI 11-30). Infection was seen in 17 patients (25%, CI 14-36). Blood was given to 61 patients (90%, CI 82-97) with a median of 4 units (0-18). Pregnancy induced hypertension was observed in 8 patients (18%, CI 4-20) while 5 patients (7%, CI 1-14) had preterm labor. One patient developed eclampsia and 1 had uterine rupture. Only 1 patient died due to post partum hemorrhage. The multivariable logistic regression model showed that none of the factors included were statistically significant on the impact on the major maternal complications composite outcome. **Conclusions.** Patients with SCD can go through pregnancy with low mortality and pregnancy-related morbidity. However, SCD related morbidity and the need for blood transfusion remain high. Baseline variables don't predict SCD and pregnancy related complications. The study is limited by the small sample size and the retrospective nature of the design. Further larger prospective studies are needed to confirm the finding.

0414

EVALUATION OF SUBCLINICAL CENTRAL NERVOUS SYSTEM INVOLVEMENT IN THE THROMBOPHILIC CONTEXT OF NON TRANSFUSION DEPENDENT THALASSEMIA SYNDROMES

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Backround. Beta thalassemia is known to be characterized by a hypercoagulable state, especially in thalassemia intermedia patients. Bibliographically, thalassemia intermedia patients also present with subclinical central nervous findings on neuroimaging examination, findings correlated to increasing age, negative thansfusion history and low levels of hemoglobin. **Aims.** To evaluate subclinical involvement of central nervous system in young non transfusion - dependent thalassemic patients, as well as frequency of coagulation abnormalities and a possible correlation of the two entities. **Methods.** Thirty one patients aged 4-21 years (mean age 12±4.7 years) participated in the study, 25 patients (Group A) with thalassemia intermedia and 6 patients (Group B) with haemoglobin H disease. All patients underwent inherited and acquired coagulation defect testing, neuroimaging and neurophysiologic evaluation. Patients aged 6-16 also had intelligence scores measured. **Results.** With regards to coagulation, a decrease in protein C and protein S activity was found in 13/25 (56%)

and 12/25(48%) of patients in Group A, respectively. In Group B 2/6 patients also had decreased protein S activity. Increased D-dimers, thrombin-antithrombin complex (TAT) and prothrombin fragment (F1 + 2) values were found in 2/25, 16/25 and 2/25, of patients in Group A and 2/6, 5/6 and 2/6 patients of Group B, respectively. Heterozygosity and homozygosity for the methylenetetrahydrofolate reductase mutation was found in 12/25 and 3/25 of patients in Group A and 3/6 and 0/6 of patients in Group B, respectively. Heterozygosity for factor V Leiden and G20210FII was found in 2/25 (8%) and 3/25 (12%) of patients in Group A, with increased prevalence compared to Greek population. Neuroimaging evaluation was normal in all patients. Neurophysiologic evaluation revealed abnormal findings in 9/25 of patients in Group A and 1/6 patients in Group B on electroencephalogram (EEG), 4/25 in Group A and 1/6 patients in Group B on brain auditory-evoked potentials (BAEPs) and 1/21 in Group A and 2/6 patients in Group B on somatosensory evoked potentials (SEPs). Visual-evoked potentials (VEPs) were normal in all patients. Significantly lower protein C values were found in patients with abnormal EEG of Group A (p=0.012). Transcranial Doppler (TCD) measurements revealed increased peak systolic velocities in 19/21 patients in Group A and 4/6 patients in Group B and decreased mean velocities in 7/21 patients in Group A and 3/6 patients in Group B. Patients with pathological findings on TCD study in Group A had significantly increased levels of F1+2 (p"□="□0.015) compared to patients with normal measurements. With regards to intelligence scores 2/18 of patients in Group A and 2/6 patients in Group B demonstrated IQ below 85. Additionally, 6/18 of patients in Group A and 1/6 patients in Group B presented mild neuropsychologic dysfunction. **Conclusions.** The study results confirm the early presence of hemostatic changes in young patients with non transfusion-depended thalassemic syndromes. Additionally, they demonstrate subclinical CNS involvement starting at childhood. For such involvement detection, in addition to commonly performed neuroimaging, neurophysiological and neuropsychological evaluation is warranted.

0415

NEONATAL CORD BLOOD SCREENING: CORRELATION OF HB BARTS QUANTITATION WITH GENESCAN AND MOLECULAR STUDIES

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Background. Alpha-thalassaemia is highly prevalent in the Sultanate of Oman. Presence of Hb Barts in significant amounts in a neonate is pathognomonic for the presence of underlying alpha thalassaemia gene. **Aims.** To correlate significantly high Hb Barts levels obtained by quantitative high performance liquid chromatography [HPLC] in the neonatal cord blood screening programme with Gene Scan studies and validate by molecular characterization of alpha thalassaemia. **Materials and Methods.** In a prospective study, consecutive cord blood samples from 7837 neonates, which showed abnormally high Hb Barts by a quantitative high performance chromatography (alpha thalassaemia short program, Biorad Laboratories, Hercules, CA, USA) were screened with Genescan studies. Samples with a single peak, (suggestive of a deletion lesion) were subjected to sequencing by GAP PCR for α2 and α1 genes. Samples that showed two peaks, (indicative of a non-deletional lesion) were initially screened for common defects like alpha T-Saudi by Fast PCR. If negative, these samples were then studied for rarer non-deletional defects by direct sequencing for **α**2 and **α**1 genes using ABI 3100 Genetic Analyzer®. (Applied Biosystems, Foster City, CA, USA).

Table 1. Distribution of Hb, Red cell indices & alpha genotype in the three subgroups of Hb Barts.

Hb Barts		Hb g/dl	RBC x10^{12}/L	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	HbF %	Genotype
<1% N= 625	Mean ± SD 0.9 ± 0.48 IQRange:0.7-1.1	15.81 ±1.58	4.54 ±0.52	49.92 ±5.03	105.72 ±5.64	34 .0 ±2.27	33.03 ±1.23	16.27 ±1.24	89.55 ±10.2	(αα/αα)
1 -3% N= 1847	Mean ± SD 1.4 ± 0.42 IQRange:1.2-1.7	15.78 ±2.03	4.74 ±0.66	48.25 ±6.99	101.57 ±5.44	33.38 ±2.69	32.71 ±1.17	16.25 ±6.46	84.65 ±9.75	(αα/−α$^{3.7}$)*
3 -10% N= 1323	Mean ± SD 5.6 ± 2.1 IQRange:4.3-7.2	14.96 ±2.01	5.29 ±0.69	47.46 ±6.8	89.87 ±5.39	28.47 ±2.1	31.65 ±1.39	17.3 ±1.82	81.42 ±9.5	(−α$^{3.7}$/−α$^{3.7}$)#

* 5.9% of these subjects had additional non-deletional mutation increasing the Hb Barts level.
# 5.6% of these subjects had additional non-deletional mutation increasing the Hb Barts level.

Results. Overall there were 3795 samples (48.42%) with Hb Barts indicative of the presence of α-thalassaemia. Amongst these, 3170(83.5%) subjects showed a significant amount of Hb Barts (>1%) ; with Genescan studies showing one peak in 1229 cases(38.77%) and two peaks in 1941 cases(61.23%). These results showed a 100% concordance with GAP PCR for α2 and α1 genes showing deletional (-α3.7/-α3.7) and non-deletional type (αα/-α3.7) in subjects with one and two peaks respectively. Furthermore, Hb Barts levels in the three groups also correlated significantly with red cell parameters and the alpha genotype (Table 1)**. Conclusions.** The amount of Hb Barts in neonates was proportional to the number of deletional **α** thalassaemia alleles. Furthermore, additional non-deletional mutation was associated with a further rise in Hb Barts. Genescan and DNA sequencing by GAP PCR showed a good correlation with elevated Hb Barts in the neonate.

0416

SAFETY AND EFFICACY OF HYDROXYUREA IN CHILDREN AND ADOLESCENTS WITH SICKLE/BETA-THALASSEMIA: THE GREEK EXPERIENCE

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Background. Hydroxyurea is a cytotoxic, antimetabolic and myelosuppresive drug that has been used over the last years in adult patients with sickle cell disease (SCD), resulting in reduction in pain crises, hospitalizations and need for transfusions. Hydroxyurea has also been used, during recent years, in the treatment of children with severe SCD. However, data on young patients with a mild clinical course, such as usually present in sickle/beta-thalassemia (S/b-thal), is limited. **Aims.** To evaluate the safety and efficacy of hydroxyurea in Greek children and adolescents with Sthal. **Patients and Methods.** 13 patients (8 girls and 5 boys) with thal, aged 9.4±4.5 years (3.5-18), were included in the study. Hydroxyurea's daily dose ranged from 15-25 mg/kg, with a mean of 18.6 mg/kg. Treatment was given for 12 months. Laboratory follow-up consisted of white blood cell count, hemoglobin, hematocrit, red blood cell indices, reticulocyte count and platelets count measured every 2 weeks, biochemistry measured every 2 months and hemoglobin F measured every two to three months. Patients were clinically evaluated on every visit. Clinical course and adverse events were reported for the study period. **Results.** A reduction of pain crises as compared to the year before treatment (median:2, 0-5 vs median:0, 0-3, p=0.003) as well as of hospitalizations (median:1, 0-3 vs median:1, 0-1, p=0.014) was noted. None of the patients presented with a severe clinical event (acute chest syndrome, avascular bone necrosis, stroke, splenic sequestration crisis) during the study period. With regards to hematological parameters, an increase in hemoglobin (9.2±0.7 vs 9.7±0.8 g/dl, p=0.048), fetal hemoglobin (9.1±5.9 vs 23.4±8.9 %, p<0.001), mean corpuscular volume (66.5±3.7 vs 86.5±9.5 fl, p<0.001) and mean corpuscular hemoglobin (21±1.1 vs 27.5±2.9 pg, p<0.001) and a decrease in reticulocyte count (9.4±4.5 vs 4.2±2.3 %, p<0.001), white blood cell count (9854±3402 vs 7607±3401 k/ul, p=0.013), platelet count (382153±184509 vs 290307±135675 k/µl, p=0.005), total bilirubin (2.2±1.4 vs 1.3±0.6 mg/dl, p=0.019) and indirect bilirubin level (1.8±1.2 vs 1±0.5 mg/dl, p=0.016) was noted. With regards to adverse events, 2 patients had mild transaminasemia, 2 had mildly elevated serum creatinine levels, one transient thrombocytopenia and one transient leukopenia with neutropenia. All of the above mentioned toxicities were short-term and dose-dependable. **Conclusions.** To the best of our knowledge, this is the first study to specifically assess the effect of hydroxyurea therapy in young patients with S/b-thal and of the same ethnic origin. The study shows that hydroxyurea treatment is safe and efficacious in this patient cohort, however, long-term follow up and evaluation of possible protective effect on organ dysfunction is warranted.

0417

THE ROLE OF ULTRASOUND IN DIFFERENTIATING ACUTE OSTEOMYELITIS FROM VASO-OCCLUSIVE CRISIS IN CHILDREN WITH SICKLE CELL DISEASE

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Background. In the acute stage, vaso-occlusive sickle crisis and osteomyelitis present with similar symptom such as pain, tenderness and swelling over the affected area with or without a fever; there may also be an associated rise in laboratory markers of infection such as white cell count (WCC) and C-reactive protein (CRP), without an obvious source of infection. These non-specific signs make distinguishing between both conditions a challenge for clinicians. Prompt diagnosis and treatment of osteomyelitis with surgical incision and drainage of affected sites when indicated, and extensive antibiotic therapy is crucial to prevent further complications. However, incorrect diagnosis of osteomyelitis in a child with vaso-occlusive crisis will result in unnecessary antibiotic treatment

and lengthy inpatient admission. The gold standard for diagnosing osteomyelitis is a positive blood culture or joint aspirate, however blood cultures are not always positive and joint aspiration is a rather invasive procedure which can be difficult to undertake in paediatric patients. **Aims.** The aim of this study was to examine the use of ultrasound as a tool in aiding early diagnosis in children with sickle cell disease presenting acutely with non-specific symptoms such as bone pain, fever or swelling which are typical of acute osteomyelitis or vaso-occlusive crisis arising due to bone infarction. **Methods.** We reviewed the clinical notes, radiological and laboratory reports of all children with sickle cell disease who were admitted to our department from October 2003 to December 2010 with a diagnosis of osteomyelitis or vaso-occlusive crisis based on symptoms at presentation and results of radiological and laboratory investigations. Thirty-seven cases with osteomyelitis and fifty-eight cases with vaso-occlusive crisis were reviewed. **Results.** A positive ultrasound finding for diagnosis of osteomyelitis was defined as evidence of periosteal elevation and/or fluid collection. A negative ultrasound finding was described as normal with no evidence of periosteal elevation or fluid collection. Positive ultrasound findings were observed in twenty-eight of the thirty-seven patients (76%) with osteomyelitis who had an ultrasound examination on day 0 - 6 of admission. Periosteal elevation was ≥4mm in nine of the twenty-eight (32%) patients with positive findings. Nine of the thirty-seven patients (24%) in the osteomyelitis group had a negative initial ultrasound scan; three of these patients had repeat ultrasound scans between days 4 - 7 of admission, all of which showed subperiosteal fluid collections ≥4mm in depth which is consistent with a diagnosis of osteomyelitis. Overall, ultrasound scan was positive in thirty-one out of thirty-seven patients (84%) in the osteomyelitis group. Initial ultrasound findings were negative in 91% of patients with vaso-occlusive crisis. **Conclusions.** Ultrasound is a useful, cost-effective diagnostic modality which is relatively easy to perform; it should be one of the first-line investigations in children where there is initial clinical confusion regarding the diagnosis of acute osteomyelitis or vaso-occlusive crisis. In cases where ultrasound is negative or inconclusive and clinical concern prevails, we would suggest a repeat ultrasound and/or MRI scan to guide the diagnosis.

0418

THE XMNI POLYMORPHISM AND BCL11A SNPS MAY HELP PREDICT HYDROXYUREA RESPONSE IN IRANIAN β-THALASSEMIA PATIENTS

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Background. Hydroxyurea (HU), a drug which can reactivate fetal hemoglobin (HbF) production, is frequently prescribed to β-thalassemia patients. However, transfusion requirements of only a subset of patients are reduced upon HU treatment. Because of its side-effects, targeted prescription of HU is imperative. **Aims.** The aim of this study was to identify genetic markers that correlate with HU response. Determination of these markers could be beneficial for targeted drug prescription. **Methods.** We carried out a retrospective association study of single nucleotide polymorphisms (SNPs) in three HbF quantitative trait loci (QTLs)"the XmnI polymorphism, Bcl11A, and the HBS1L-MYB intergenic region"with the response to HU in a cohort of 89 transfusion-dependent Iranian β-thalassemia patients. An increase in blood transfusion intervals post-treatment was used as a basis for drug response. **Results.** The XmnI T/T genotype and presence of the Bcl11A rs766432 (and rs4671393) minor alleles correlated strongly with the response to HU (P<0.001). This correlation was even stronger upon presence of both markers [P<0.001; odds ratio (95% confidence interval) = 40.8 (10.0-166.4)]. **Conclusions.** The XmnI polymorphism and Bcl11A SNPs may be used to accurately predict the HU-response of Iranian β-thalassemia patients.

0419

HAEMOGLOBINOPATHY SCREENING AND PREVENTION IN NORTHERN GREECE. A 10 YEARS SURVEY

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Greece is a Mediterranean country with a high frequency of thalassaemia and haemoglobinopathies. Beta thalassaemia (β-thal) carrier frequency is approximately 8%, while 1.5% of the population is heterozygous for the HbS gene. Since 1974 a nation wide program for Thalassaemia prevention has been implemented in our country. The aim was to educate and to increase awareness of the public concerning these hereditary anaemias, to screen couples for haemoglobinopathies, to give genetic counselling and perform prenatal diagnosis. The spectrum of mutations we observe recently, is influenced by the number of immigrants from different geographic areas who have settled down in Greece the last two decades. Through population screening and prenatal diagnosis program that is performed,natives and immigrants are screened and counselled free of charge.We report the results of the prevention program in Northern Greece, over a 10-year period (2002-2011). A total of 25.520 subjects have been screened for haemoglobinopathies. Among them a total of 2602 couples were screened and counselled in our Thalassaemia Prevention Unit. In 259 cases (9,9%), both partners carried an abnormal haemoglobin gene and genetic counselling was offered. 248 out of 278 pregnancies, (including 5 twins pregnancies) were at risk of giving birth to a sick offspring carrying either the homozygous or doubly heterozygous forms of the mutations under discussion. 32 pregnancies involved couples who were heterozygous for mutations that did not cause severe clinical disease, and were exempted from prenatal diagnosis. These gene interactions were β-thal / α-thal, HbE / HbE, HbE-Saskatoon / HbS , HbE-Saskatoon / β-thal, HbO-Arab / HbO-Arab, HbD-Punjab / α-thal, HbD-Punjab / β-thal. Prenatal diagnosis was carried out by chorionic CVS at 11-12 weeks of gestation (n=218), in few cases by amniotic fluid sampling, at 16-18 weeks (n=21). Very few late-comers were tested by foetal blood sampling at 20-24 weeks of gestation (n=4), In two cases prenatal diagnosis was performed with chorionic CVS and amniotic fluid sampling and in one case with CVS and foetal blood sampling testing. In three cases a transvaginal procedure was performed. There has been one obstetric complication out of 248 procedures.It was the case of a transvaginal collection of CVS that provoked rapture of the membranes and automate abortion. 63 (26%) foetuses were found to be homozygotes or double heterozygotes for clinically significant mutations. These couples were informed of the danger of having a sick child but, as to the termination or continuation of the pregnancy, it was left to the couples to decide for themselves. Nevertheless, all the couples preferred termination of the pregnancies. Our conclusion is that, wide spread population screening for thalassaemia, in a country like Greece with a high frequency of the thalassaemia gene and that of other haemoglobinopathies, and the implementation of prenatal diagnosis in at risk couples, have both decreased the incidence of children born with Thalassaemia Major and Sickle Cell disease. This Thalassemia screening program has proved successful in our case as it has in many other countries that have implemented it.

0420

FETAL OUTCOMES IN PREGNANT PATIENTS WITH SICKLE CELL DISEASE

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Introduction. Sickle cell disease (SCD) is an inherited hemoglobinopathy with multiple complications and multisystem involvement. Fetal outcomes in pregnant patients with SCD are not well studied. We therefore looked into the fetal outcomes in a single institute tertiary care setting and analyzed the impact of baseline variables on those outcomes. **Methods.** We retrospectively analyzed the fetal outcomes of 68 consecutive pregnant SCD patients followed at the obstetric clinic in our hospital from June 2006 to August 2011. We collected different baseline variables including age, gravida, parity, previous pregnancy outcomes including intrauterine growth restriction (IUGR), preterm labor, prematurity, intrauterine fetal death (IUFD), abortions, low birth weight , maternal morbidities (eclampsia, uterine rupture and or post-partum hemorrhage) baseline hematological parameters, history of splenectomy and cholecystectomy. We used multivariable logistic regression to estimate and adjust the impact of baseline variables on major fetal complications. **Results.** We analyzed 68

patients. The mean age was 30 years (standard deviation [SD] 3.8, range 22-40). The median gravidity and parity were 2 (range 1-6) and 1 (range 0-5) respectively. Mean gestation age was 37 weeks (SD 1.88, range 32-41)Sixty-two patients had SS genotype. The initial mean hemoglobin was 9.5 g/dl (SD 1.1, range 7.2-11.9). The mean baseline hemoglobin F was 10.2% (SD 6.6, range 0.7-29). None of the patients were on Hydroxyurea during pregnancy, but eight patients were on Hydroxyurea prior to pregnancy. Twelve patients had prior cesarean section. Only one patient had previous preterm labor, none had previous premature babies, five women had previous IUFD, no IUGR reported previously.Eleven cases (16%) of IUGR were noted (95% confidence interval [C.I]:0.07-0.25, 19 cases of fetal distress (28%; 95%CI: 0.16-0.38) Five cases (7%) had elective cesarean section and 24 (35%) needed emergency cesarean section.Low birth weight was seen in 22 cases (32%, 95% C.I: 0.21-.44) with a mean weight of 2.6 Kg (SD 0.47, range 1.2-3.92. There were two neonatal deaths.Sixteen newborn babies required admission to neonatology intensive unit (23.5%). On multivariate logistic regression for a composite of fetal outcomes that included IUGR, low birth weight, perinatal mortality and admission to neonatology units, none of those variables was of statistical significance. **Conclusions.** Our study shows that the adverse fetal outcomes in pregnant patients with sickle cell disease are high despite the fact that they were followed up a tertiary care centre with high risk obstetric care clinic along with specialized haematology follow up.We where not able to identify significant difference in the fetal outcome between SCD, SS genotype versus others likely owing to small number of other subtypes. Larger sample size may help to show if there is any significant difference.

0421

IMPORTANCE OF DIAGNOSIS OF NON-DELETIONAL ALPHA-THALASSEMIA

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Background. The molecular basis of α-thalassemias are mainly deletions in α-globin genes, however, about 5-10% of cases are due to mutations in these genes. Most of these mutations are exclusive to each family, but some mutations as Nco and Hph are more common in some populations. Diagnosis of less frequent variants of this disease is essential in order to be applied to genetic counseling and prenatal diagnosis. **Aims.** In this paper we present the cases of non-deletional α-thalassemia diagnosed in our department from January 2009 to December 2011. **Methods.** Between January 2009 and December 2011 were studied 1127 patients collected by our center for study of α-thalassemia by presenting microcytosis with normal HbA_2 and HbF and without iron deficiency. The geographical origin of patients covered all the spanish territory.Molecular study required the extraction of genomic DNA. Deletions were ruled by α-globin StripAssay. The rest of patients were studied by automatic sequencing specific for both α_2 and α_1 genes.Results606 of the patients had α-thalassemia, of which 544 showed deletions (89.77%) and 62 patients (10.23%) mutations. Point mutations are set out in Table 1.

Table 1. Summary of point mutations in both 2 and 1 genes that are responsibles of a non-deletional alpha-thalassemia phenotipe in our study.

MUTATION NAME	AFFECTED GENE	GENE LOCATION	NUMBER OF CASES
Hph	α_2	IVS I -5nt	23
Hb Agrinio	α_2	CD29 CTG→CCG (Leu→Pro)	11
Hb Groene Hart	α_1	CD119 CCT→TCT (Pro→Ser)	11
CD23	α_2	CD23 GAG→TAG (Glu→AMB)	4
nt+778 C→A	α_1	nt+778 C→A	3
IVS-I-38 C→T	α_1	IVS-I 38 C→T	2
Hb Q-Thailand	α_1	CD74 GAC→CAC (Asp→His)	2
Hb Plasencia	α_2	CD125 CTG→CGG (Leu→Arg)	1
IVS-I-1 G→A	α_2	IVS I-1 (G→A)	1
T-Saudí	α_2	PolyA AATAAA→AATAAG	1
Constant Spring	α_2	CT TAA→CAA (Stop→Gln)	1
Hb Tunis-Bizerte	α_1	CD129 CTG→CCG (Leu→Pro)	1
Poly A	α_2	PolyA AATAAA→AATGAA	1

Conclusions. The identification of 13 different point mutations variants reveals the molecular heterogeneity of non-deletional α-thalassemia cases in our environment. This heterogeneity may have important implications in terms of its phenotypic expression, to be able to interact with other forms of deletional and non-deletional α-thalassemia more common and that are increasing in our country by migratory changes.However, five variants (Hph, Hb Agrinio, Hb Groene Hart, CD23 and nt +778 C>A) reflects the 84.07% of cases of non-deletional alpha-thalassemia. This fact has been seen in studies on other populations. For example, in Iranian population three variants (Hph, Hb Taibe and T-Saudí) account for 83.26% of non-deletional alpha-thalassemia collected cases.Integration of different molecular techniques, especially the automatic sequencing, is of great importance for the clinical diagnosis of thalassemia syndromes, mainly in the rare cases of non-deletional α-thalassemia. However, if variants mentioned above will be introduced in the available screening methods, it would allow an improved initial screening, which would result in a economic-saving and less time-consuming diagnosis.

0422

VITAMIN D STATUS IN PATIENTS WITH THALASSAEMIA MAJOR AND INTERMEDIA: ITS CORRELATION WITH IRON STATUS AND IMPACT ON OUTCOME

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Background. Vitamin D deficiency is common in patients with thalassemia. However, data on its impact on outcome and its correlation with iron load is limited. **Aims.** We aimed to investigate the prevalence of vitamin deficiency and insufficiency in patients with thalassemia major and intermedia and the study relationship between vitamin D status and iron overload. In addition, we planned to report the impact of vitamin D status on bone health in these patients. **Methods.** 25OH vitamin D (D25OH), albumin adjusted calcium, phosphate, alkaline phosphatase, parathyroid hormone (PTH) and ferritin levels were measured on all patients with thalassemia major and intermedia who are being followed at our institution. Data on age, gender and body mass index (BMI) were collected as well as data on cardiac T2* and liver iron concentration by MRI (LIC), bone density measurement (BDM) and fractures. We classified patients depending on their vitamin D levels into: sufficient (>50 nmol/L), insufficient (25-50nmol/L) and deficient (<25 nmol/L). We used multivariate ordinal regression to study the relation between vitamin D status and ferritin, cardiac and liver iron status, gender, BMI and BDM. **Results.** A total of 123 patients with thalassemia major (106) and thalassemia intermedia (17) were evaluated. There were 64 females and 59 males. Median age was 23±7.9 years (range:5-58) with mean BMI of 21.5±4.8 (13.7-37.2), median ferritin 1182 ng/mL (not normally distributed, range: 76-10500), cardiac T2* 30.3±13 (5.5-60) and LIC 7.1±6.7mg/g dry weight (1-32). Nine patients had insulin dependent and 9 had non-insulin dependent diabetes mellitus while 27 patients had hypoparathyroidism. Mean D25OH was 48.8±23nmol/L (12-131) with 47% having vitamin D insufficiency and 12% vitamin D deficiency. Hypocalcemia was seen in only 3 patients and hypophosphatemia in 2 patients. Of 63 patients over the age of 20 who had BDM, 25 (40%) had osteopenia (lumbar Z score and/or femoral Z score <-1.0 to >-2.5): 3 vitamin D deficient and 13 insufficient, while 32 patients (51%) had osteoporosis (lumbar Z score and/or femoral Z score <-2.5): 4 deficient and 7 insufficient . Four patients had fractures: 2 vertebral and 2 extremity; 1 in the osteopenic group, 1 in the osteoporotic group and 2 in patients less than the age of 20. Three of the 4 fractures occurred in patients with vitamin D deficiency and/or insufficiency. On the multivariate ordinal logistic regression, only ferritin had a significant correlation with vitamin D status (OR 1.2; 95% CI 1.1-1.3; p 0.001). There was no significant correlation between vitamin D status and cardiac or liver iron load. In patients over 20, there was no significant correlation between vitamin D status and BDM status. **Conclusions.** Vitamin D deficiency and/or insufficiency state is common in patients with thalassemia with a significant correlation with ferritin levels. Its contribution to osteoporosis and osteopenia requires further studies. The impact of vitamin D replacement in patients with thalassemia major and intermedia needs to be studied prospectively.

0423

SAFETY AND EFFICACY OF ERYTHROPOIETIN IN PATIENTS WITH SICKLE CELL ANEMIA AND CHRONIC RENAL INSUFFICIENCY

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Background. The use of recombinant erythropoietin (epo) in sickle cell anemia (SCA) is controversial. However, use of epo to improve hemoglobin in patients with renal failure is well established and renal failure is common in SCA. Because SCA results in increased filtration, renal function is often worse than it appears based on serum creatinine. Furthermore, some authors have found an increase in fetal hemoglobin in patients with SCA after administration of epo. Therefore, the use of epo in patients with SCA and chronic renal failure is attractive, although there are no randomized controlled trials definitively demonstrating benefit. **Aims.** To examine the safety and efficacy of epo usage in SCA and renal insufficiency. **Methods.** Charts of patients at the Thomas Jefferson University hematology clinic who were treated with epo for SCA with low reticulocyte count thought to be due to chronic renal insufficiency were reviewed. Data were extracted including hemoglobin and reticulocyte count at baseline and after treatment with epo, serum creatinine, pre-treatment erythropoietin level, and clinical status. Hemoglobin F level was recorded, when available. **Results.** Six patients received weekly or semiweekly recombinant erythropoietin or darbepoietin injections. Five of the six took hydroxyurea (HU). One did not due to intolerance. All patients had hypoplastic anemia with decreased baseline hemoglobin, with mean hemoglobin of 6.2 gm/dl, range of 5.5-7.9 gm/dL and low baseline reticulocyte count, mean 178,000 cells/µL, range 109-272,000 cells/µl. All patients had adequate iron stores as assessed by serum iron, iron binding capacity, and ferritin levels. All responded to epo administration with an increase in hemoglobin (mean hemoglobin on erythropoietin 9.1 g/dl, range 6.7-11.3) and reticulocyte count (mean on epo 204,000 cells/µl, range 140-273,000 cells/µl). Mean creatinine was in the normal range at a mean of 1.1 mg/dl (range 0.3-1.7 mg/dl). Baseline erythropoietin levels were elevated at a mean of 81.3 mIU/ml, but lower than expected for the degree of anemia seen. Fetal hemoglobin was measured both pre- and post-erythropoietin in two patients and no change was seen for either patient. Two patients who were transfusion dependent prior to starting epo no longer required regular transfusions after treatment. The patient not taking HU suffered a stroke while on therapy and therapy was changed to chronic exchange transfusions. No other patient suffered major toxicity from treatment with epo. **Conclusions.** Erythopoietin supplementation can be useful in patients with SCA and chronic renal insufficiency. Serum creatinine is an unreliable marker of renal function and may underestimate the possibility of hypoplastic anemia in patients with SCA. The decision to start epo should be made based on the overall clinical picture, including erythropoietin level, hemoglobin, and reticulocyte count. Epo should be used only with caution in patients with SCA and our experience suggests that epo should not be used in patients not receiving concurrent HU. Formal phase III studies to confirm the safety and efficacy of epo with HU in SCA with chronic renal insufficiency would be helpful for delineating the optimal timing and dosing of epo in SCA.

0424

INTRACRANIAL BLOOD FLOW VELOCITIES IN LEBANESE PATIENTS WITH SICKLE CELL DISEASE AND ?-THALASSEMIA INTERMEDIA

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Background. Thalassemia intermedia (TI) and sickle cell disease (SCD) represent two common forms of hereditary anemias that share many clinical manifestations in developing cerebral infarctions [1, 2]. Transcranial Doppler ultrasonography (TCD) detects patients with SCD who are at an increased risk of developing infarction but its role in identifying high risk patients with TI has not been evaluated [3]. **Aims.** The aim of this study is to compare the mean maximal cerebral blood flow velocities (MBFV) in patients with SCD and TI. **Methods.** Between January 2011 and October 2011, a total of 60 patients with SCD and 20 patients with TI were examined. After obtaining informed consent, the right and left, middle and anterior cerebral arteries (RMCA, LMCA, RACA, LACA) were insonated using a temporal window approach. The MBFV of each artery was recorded separately. Demographic and recent laboratory information was collected for each patient. **Results.** Table 1 shows the baseline characteristics of the 2 groups of patients along with the averages and standard deviations of MBFV in the measured arteries. The 2 groups were matched for sex and hemoglobin concentration. However, TI patients were significantly older. Fifty-four out of the 60 sickle cell patients have SS (90%) while 6/60 (10%) have Sβ°, and 48/60 (80%) were on hydroxyurea. MBFV in the RACA was significantly higher in the sickle cell patients (p = 0.006). This difference was borderline significant in the case of the LMCA (p = 0.056), and not significant in the RMCA and LACA.**Conclusions**This is the first report to compare MBFV in TI and SCD patients. Sickle cell patients have been found to have higher MBFV than the normal population in several studies [3, 4]. Several mechanisms have been implicated including anemia, deficiency of nitric oxide synthesis, and endothelial dysfunction. The pathophysiology of SCD and TI includes disruption of these mechanisms. In this study, the mean MBFV values in 20 TI patients were lower than those of 60 SCD patients, despite similar hemoglobin levels, yet they were still higher than those reported for the normal population [5]. The difference was statistically significant for the RACA despite the fact that 48/60 (80%) of our SCD patients were on hydroxyurea treatment which is known to lower MBFV values. Larger scale studies with longer follow up are warranted to validate these findings and define any risk in the TI population.

Table 1. Baseline characteristics of 20 TI and 60 SCD patients with their measured maximal cerebral blood flow velocities.

	Thalassemia Intermedia	Sickle Cell Disease	p-value
N	20	60	
Age	28.0 ± 11.1 [10 – 47]	13.8 ± 8.2 [2 – 42]	p<0.01
Sex (F/M)	13/7	32/28	p=0.264
Hemoglobin	8.9 ± 2.0	9.2 ± 1.2	P=0.294
Hydroxyurea treatment	-	48/60 (80%)	
RMCA	93 ± 28 [39 – 155]	106 ± 28 [55 – 173]	p=0.94
LMCA	91 ± 28 [35 – 148]	104 ± 25 [50 – 169]	p=0.056
RACA	64 ± 18 [34 – 99]	80 ± 22 [41 – 143]	p=0.006
LACA	71 ± 18 [41 – 115]	76 ± 20 [36 – 117]	p=0.395

Infectious diseases - Viral

0425

PHARMACOKINETIC ASSESSMENT OF POSACONAZOLE SUPPORTS AN EFFECTIVE ANTI-FUNGAL PROPHYLAXIS IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Posaconazole (PSC) is an effective broad-spectrum anti-fungal triazole recommended for prophylaxis of Invasive fungal diseases (IFDs) in induction of acute leukemia patients and in GVHD phases of allogeneic transplant; the pharmacokinetic of PSC is linear and absorption strongly depends on gastric pH and nutrition. The registered schedule for IFDs' prophylaxis consists of 200 mg tid and preliminary studies suggest that ideal plasma concentrations for the best efficacy should be ≥0.7 µg/mL. **Aims.** To evaluate the effect of anti-fungal prophylaxis with PSC 200 mg bid in acute myeloid leukemia (AML) patients, supported by pharmacokinetic analysis. **Methods.** Sixty-four patients (32M/32F, aged 18-75 years) treated for AML at the Haematological Unit of the Foundation S. Matteo Pavia from January 2010 to December 2011 were enrolled. All patients received a dosage of 200 mg bid during neutropenic phases of induction, consolidation and reinduction therapy and serum levels were monitored on a weekly basis (at least 4 samples for each pts). PSC was administered with fatty food, while proton pump inhibitors or H2 antagonist were avoided during the entire prophylactic treatment. Plasma samples, taken at the steady-state, just before administration of PSC and during treatment levels, were measured on HPLC-UV. In term of efficacy, we compared the incidence of IFDs occurred in studied patients with that of 93 AML patients treated in the previous biennium who didn't receive any anti-fungal prophylaxis. **Results.** We analyzed 283 plasma samples. A high variability in PSC through levels was observed. During treatment, 55/283 (19%) of measurements resulted below 0.7 µg/mL, 189/283 (67%) ≥1 µg/mL and 39/283 (14%) were ranged between 0.7 and 1 µg/mL. Patients achieving a PSC measurement ≥0.7 µg/mL were 56 (88%).Median Ctrough was 1.43 µg/ml (0.14-5.92). PSC plasma levels increased after 48 hours from the first administration (1.7±1.64 vs 0.52±0.09 p<0.001) and by food intake (1.74±1.64 *vs* 0.58 ± 0.37 p<0.001). No relationship was observed between alanine aminotransferase, aspartate aminotransferase, bilirubin or creatinine levels and PSC levels. No relevant side effects were observed. In term of efficacy we observed a significant reduction of IFDs incidence in posaconazole group with respect the cases without prophylaxis (9,2% vs 22,6% p=0.03). **Conclusions.** Posaconazole 200 mg bid resulted in median plasma drug concentrations achieving therapeutic range in almost 90% of patients with a good tolerability. Plasma levels varied in time from the initial dose and were influenced by food intake. For these reasons we support the therapeutic drug monitoring during treatment. Finally, this prophylactic schedule allowed a significant reduction of invasive fungal infection.

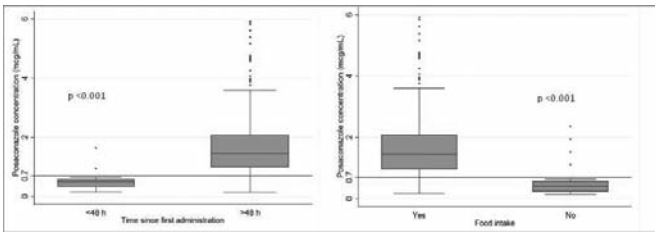

Figure 1. PSC serum level according to time since first administration and food intake.

0426

THE ROLE OF PET-CT FOR THE DIAGNOSIS OF INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND PERSISTENT FEBRILE NEUTROPENIA

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Background. Patients with hematological malignancies and prolonged febrile neutropenia are at high risk for bacterial and invasive fungal infections (IFIs). **Aims.** We aimed to evaluate the role of PET-CT for detection of such infections among these patients. **Methods.** Prospective cohort study of patients with hematological malignancies given intensive conventional chemotherapy and hematopoietic-cell transplantation (HCT). All consecutive, consenting patients with neutropenia (<500/mm^3) and persistent or breakthrough fever despite broad spectrum antibiotics (>5days) had a PET-CT examination. The CT component of the PET-CT was a contrast-enhanced diagnostic CT. Blinded evaluation of chest and sinus CT and the full PET-CT scan were compared with the final clinical diagnosis 30 days after neutropenia resolution, as determined by an expert panel consisting of a hematologist and an infectious diseases expert. **Results.** Between January 2008 and January 2011, 91 PET-CT examinations were performed in 79 patients. Median age was 56 (range: 21-85) years. PET-CT was performed after a median of 10 days from last chemotherapy (range: 0-255). Patients were neutropenic for a median of 11 (range:1-100) days.Most patients had acute leukemia (71 episodes), 7 patients underwent allogeneic HCT and 6 patients with lymphoma underwent autologous HCT.The types and number of individual diagnoses are listed in the table: Of the 91 PET-CT examinations, 23 episodes had two or more diagnoses, most commonly a combination of bacterial and fungal infection. Of 28 microbiologically documented infections (MDIs), bacteremia was the diagnosis in 20 episodes, most commonly without a focal source. Fifteen episodes were classified as fever of unknown origin.The sensitivity to detect any infection or non-infectious pathology in chest/sinus CT, was 58.8% (60 /102 diagnoses). The respective sensitivity for-PET- CTwas 85.3% (87/102). The difference in sensitivity was 26.5% (95% confidence interval 21.4% to 31.6%), matched sample p<0.001. The specificities of CT and PET-CT were not significantly different, 66.7% (10/15 episodes of FUO) and 60% (9/15), respectively. Of note, all 7 proven or probable fungal infections were FDG- positive.In 28 cases, PET-CT demonstrated findings which were not detected on chest/sinus CT (27.5% of diagnoses). These were mainly abdominal infections (as appendicitis, diverticulitis, etc.) and abscesses (perianal, splenic, etc.).When we compared PET-CT to total body (chest, sinus and abdomen) CT, we found that 7 of these cases were found only on PET-CT. The sensitivity of total body CT to detect disease was 78.4% (80/102).PET-CT resulted in modifications of patients' management in 46 (55%) cases. These included change in antibiotics (14 cases), change in antifungals (14), change in both (5), an invasive diagnostic procedure (7), a surgical procedure (i.e appendectomy, 3) and abscess drainage (4). **Conclusions.** PET-CT has a higher sensitivity with no loss of specificity compared to chest/sinus CT in patients with persistent febrile neutropenia. The increase in sensitivity afforded by PET-CT was mainly due to the addition of abdominal CT.

Diagnostic Category	Number of diagnoses (total 117)	Positive findings on PET/CT (positive findings with FDG uptake)	Positive findings on chest/sinus CT
Fever of unknown origin =no disease	15	6 (3)	5
Disease (Infectious causes):			
• Clinically documented infection (CDI)	34	31 (25)	14
• microbiologically documented infection (MDI)	28	16 (13)	9
• Possible invasive fungal infection (IFI)	20	20 (16)	19
• Probable/proven IFI	7	7 (7)	7
Disease due to primary malignancy or non-infectious causes	13	13 (10)	12

Table 1. Characteristics of diagnoses.

0427

AT HOME TREATMENT AFTER HIGH DOSE CHEMOTHERAPY IS SAFE AND FEASIBLE, AND LEADS TO SIGNIFICANT COST SAVINGS

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Background and aim of the study. The necessity of keeping patients in hospital during neutropenia after high dose chemotherapy is under discussion. A prospective, nonrandomized, single center clinical study was performed to examine the safety and feasibility of ambulatory care in patients undergoing consolidation chemotherapy for acute leukemia, or autologous stem cell transplantation following high dose chemotherapy for lymphoma or multiple myeloma. In addition, patient satisfaction, costs and use of hospital resources were evaluated. **Patients and methods**. Patients fulfilling the eligibility criteria were discharged into ambulatory care the day after the last chemotherapy administration, or the day after reinfusion of the stem cells. Eligibility criteria included, but were not restricted to: adequate performance status, adequate fluid intake, understanding of risks and procedures, availability of a caregiver and travel time to the hospital <1 hour. Patients visited the ambulatory care unit 3 times a week for monitoring of signs, symptoms and laboratory results. Patients were asked to fill out a questionnaire with 21 questions on logistics, information, commu-

nication and patient perception.*Results*During the study period, 165 patients were admitted for 208 chemotherapy cycles. 76 patients in 89 cycles could not be included in the ambulatory care program, most frequently either because their medical situation did not allow for early discharge (58%), or because they had no care giver (15%), or had to travel a large distance to the hospital (15%).The 89 patients in the ambulatory care group (37 AML, 20 NHL, 17 ALL, 12 MM, 3 Hodgkin lymphoma), who underwent 119 cycles of high dose chemotherapy, spent almost 70% of the neutropenic phase at home. 37 out of 89 patients (46 cycles) were never readmitted to the hospital. None of the patients in the ambulatory care group had to be admitted to the intensive care, and there was no treatment related mortality. The most frequent reason for readmission was fever, and the median duration of readmission was 6 days (range,1-21 days). In the hospital group, 2 patients died, one because of pneumonia and one because of invasive aspergillosis. Ambulatory care led to significant cost savings, also when corrected for visits to the day care unit, and increased the capacity for admissions to the hematology department. Patients and their caregivers felt safe and comfortable at home, and the vast majority preferred home care over in-hospital treatment and would recommend it also to other patients. **Conclusions**. This study demonstrates the safety and feasibility of managing carefully selected patients in an ambulatory care setting after high dose chemotherapy. In addition, ambulatory care leads to significant cost savings, more efficient use of hospital beds, and a high degree of patient satisfaction.

0428

CLINICAL AND MICROBIOLOGICAL ASPECTS OF DISCONTINUATION OF QUINOLONE PROPHYLAXIS IN HAEMATOLOGICAL PATIENTS WITH CHEMOTHERAPY-INDUCED NEUTROPENIA: A SINGLE-CENTRE EXPERIENCE

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Background. Routine use of quinolone prophylaxis in neutropenic haematological patients is widespread. Although it has been reported to result in significant survival advantages, emergence of bacterial resistance has become a concern. **Objectives** The aim of this study was to evaluate the clinical and microbiological impact of discontinuing ciprofloxacin prophylaxis (CP) in haematological patients with prolonged neutropenia. **Methods**. Between August 2009 and July 2011 a total of 269 consecutive admissions for polychemotherapy treatment followed by prolonged neutropenia were evaluated in this retrospective study. 43 were excluded due to documented infection or antibiotic treatment in the week prior to the start of treatment. Three sequential periods of 8 months were analysed: on-going routine use of CP, discontinuation of CP and reintroduction of CP. Patient characteristics were comparable between groups. Clinical endpoints were occurrence of neutropenic fever, bacteraemia, severe sepsis, septic shock and infection related mortality. Microbiological analysis included inventarisation of bacterial isolates from stool and blood cultures and their resistance pattern. Total per protocol antibiotic use for treatment of neutropenic fever and duration of hospitalisation were also analysed. **Results.** The incidence of neutropenic fever was significantly lower in patients receiving CP (55.1% versus 69.1%; p=0.049). However, the incidence of bacteraemia, severe sepsis, septic shock and infection related mortality did not differ significantly between groups. Routine microbiological screening of stools showed a higher prevalence of pathogens in the group not receiving CP (87.2% versus 47.0%; p<0.001). However, these were more frequently multisensitive (56.1% versus 10.9%, p<0.001). After CP discontinuation quinolone resistance disappeared very rapidly (7.3% versus 72.7%; p<0.001) with also a clear decrease in the presence of ESBL producing isolates (9.8% versus 34.5%; p=0.005). As stated before the overall incidence of bacteraemia did not change with discontinuation of CP, but there was a significantly higher proportion of gram-negative blood culture isolates (55.5% versus 21.1%; p=0.009). But again these isolates more often showed a multisensitive pattern (54.5% versus 0%; p=0.012) and less quinolone resistance (9.1% versus 100%; p<0.001). Antibiotic consumption and duration of hospitalisation did not differ significantly between groups. **Conclusions.** Because of increasing quinolone resistance, routine ciprofloxacin prophylaxis in patients with chemotherapy-induced neutropenia was discontinued. This did not lead to an increase in serious infectious complications nor to an increase in antibiotic consumption, although there was an increased incidence of neutropenic fever. Quinolone resistance disappeared very rapidly after discontinuation of ciprofloxacin prophylaxis and also reappeared very quickly after reintroduction. Analysis of this data has led us to suspend routine ciprofloxacin prophylaxis indefinitely.

0429

RECURRENCE OF BLOODSTREAM INFECTIONS AMONG ACUTE LEUKAEMIA PATIENTS DURING CONSECUTIVE TREATMENT: IMPACT ON EPIDEMIOLOGY AND OUTCOME

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Introduction. Antibiotic prophylaxis with fluoroquinolones (Fq) in Acute Leukaemia (AL) patients (pts) receiving chemotherapy is a well established practice. Nevertheless, some pts experience more than one episode of bloodstream infection (BSI) caused by the same pathogen during the entire program of chemotherapy. **Aims.** In order to better define the actual risk of developing BSI recurrence (BSI-R) during subsequent phases of treatment, we analyzed all BSI occurring to consecutively treated AL pts at our Institute during a period of 7 years. **Patients and Methods.** Since June 2004 a program of active epidemiological surveillance is ongoing at our Institute. Data concerning infections occurring during chemotherapy-induced cytopenia in AL pts were analysed. All pts showing fever or signs/symptoms of infection underwent at least two sets of blood culture. **Results.** From June 2004 to May 2011, 258 cases of AL (197 acute myeloid leukaemia, 58 acute lymphoblastic leukaemia, and 3 blastic plasmacytoid dendritic cell leukaemia), were diagnosed and treated with at least one induction cycle followed by consolidation and with reinduction cycles in relapsing/refractory pts. Overall, 1263 chemotherapy cycles were delivered; 234 pts received more than 1 cycle. Two hundred and fifty BSI were observed (20.2%) in 139 pts receiving more than 1 cycle of chemotherapy (median 5, range 2-13); in 39/139 (28.1%) pts BSI recurred (median relapses: 1, range 1-4). Gram-negative (G-) bacteria accounted for 59.6% of BSI in 99 pts, Gram-positive (G+) for 34.4% in 68 pts and polymicrobic for 6% in 14 pts. Among G- BSI, *E. coli* and *P. aeruginosa* were the more frequent pathogens (115, 46% and 32, 12.8%, respectively), among G+, Coagulase Negative Staphylocci (CoNS), viridans streptococci and enterococci (38, 15.2%; 29, 11.6% and 21, 8.4%, respectively). *E. coli*, *P. aeruginosa*, CoNS and *S. viridans* were the four type of pathogens involved in recurrence. G- were significantly more frequent among BSI-R in comparison with other BSI (72.3% vs 50.6%, p =0.0009); in particular, *E. coli* accounted for 70.2% of BSI-R vs 33.3% for non relapsing BSI (p=<0.0001). All but 3 pts with BSI-R were neutropenic and previously treated with Fq prophylaxis; all relapsing pathogens showed Fq resistance (FqR). BSI-R did not correlate neither with type of AL nor with phase of disease, whereas it correlated with more than 4 delivered courses (84.6% vs 59%, p<0.0049). Fourteen out of 139 pts died within 30 days from BSI (10.1%); mortality was higher, although not significantly so, among pts with BSI-R (6/39, 15.4%) in comparison with pts without (8/100, 8%), particularly for pts with *P. aeruginosa* BSI-R (3/6, 50% vs 3/18, 16.6%). **Conclusions.** Among AL pts, BSI-R is frequent and often associated to FqR G- bacteria, particularly *E. coli*; this phenomenon is responsible for a shift towards G- epidemiology. Neutropenic pts treated with more than 4 courses were at risk for relapsing BSI. Mortality due to BSI recurrence seems to be higher in comparison with pts who do not experience it. Further observational studies are warranted in order to assess if prophylactic strategies other than Fq could be worthwhile.

0430

REVERSIBLE SKELETAL DISEASE AND HIGH FLUORIDE SERUM LEVELS IN HEMATOLOGIC PATIENTS RECEIVING VORICONAZOLE

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Background. Recently case reports of periostitis and skeletal disease in solid organ recipients with long-term voriconazole treatment have been published. **Aims.** We here investigate the occurrence of fluoride-intake associated alterations in patients with hematologic disease on triazol antifungal medication. **Methods.** We describe clinical, laboratory and radiology data of overall 43 patients with hematologic malignancies taking voriconazole (n=20), posaconazole (n=8), and itraconazole (n=4), and a hematologic patient control group (n=11). **Results.** Bone pain and radiologic evidence of periostitis was exclusively observed in patients receiving long-term voriconazole. Secession of treatment led to clinical improvement in all cases. In line with clinical evidence, fluoride serum concentration was elevated in patients receiving voriconazole (median 156.5 µg/l, interquartile range 96.8 µg/L; normal <30 µg/L) but not in the other treatment groups (p <0.001 for all comparisons against voriconazole).Summary/ **Conclusions.** Serum fluoride levels were elevated on average five-fold above normal levels in hematological patients receiving voriconazole. Clinical relevant skeletal disease was associated with

renal insufficiency and above ten-fold elevated fluoride levels, and was reversible upon termination of voriconazole treatment.

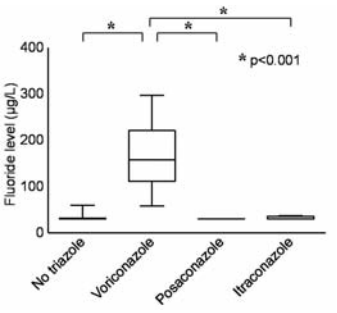

Figure 1.

0431

ASSOCIATION OF TNF-?, IL-6 AND IL-10 GENETIC POLYMORPHISMS AND IL-10 HAPLOTYPES WITH INFECTION SUSCEPTIBILITY AND OUTCOMES IN PATIENTS WITH MULTIPLE MYELOMA SUBMITTED TO ASCT

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Background. The number of onco-hematological patients undergoing stem cell transplantation (SCT) has increased significantly in recent years; however bacterial, viral and fungal infections are major obstacles to its success. SCT increases patient's vulnerability to infections due to immunological changes related to the conditioning regimen. Multiple myeloma is a plasma cell neoplasm characterized by production of a monoclonal protein, bone destruction and susceptibility to infections. The introduction of Autologous Stem Cell Transplantation (ASCT) increased the overall survival of these patients, but infection still remains an important complication of the procedure. The increased levels of cytokines expression have an important relationship with the response immune. Studies have show that these Single Nucleotide Polymorphisms (SNPs) in the promoter region of cytokine genes are responsible for altering the levels of expression, affecting the stage of the disease and the immune response. **Aims.** Evaluate the association of the SNPs in the TNF-α, IL-6 and 10 genes and their haplotypes, with the infection susceptibility. **Methods.** Genomic DNA was extracted from mobilized peripheral-blood stem cells of 148 patients from the Bone Marrow Transplantation unit of Federal University of Rio de Janeiro. Genotyping was carried out for the SNPs IL-6 (-174G/C), IL-10 (-1082A/G, -592A/C e -819T/C) and TNF-α (-308G/A) using the Real-time PCR assay and PCR followed by restriction length fragment polymorphism (PCR-RFLP). Haplotype frequencies were estimated with Haplo.stats® and all the other statistical analysis were performed with SPSS®.**Results.** To evaluate the results we find all the alleles in Hardy-Weinberg equilibrium. The TNF-α polymorphism was significantly associated with fever of unknown origin (*p=0.03*), IL-6 polymorphism was associated with fungemia (p=0.04) and death during neutropenia (p=0.02), and IL-10 polymorphism was associated with superinfection (*p=0.02*). From the studied IL-10 haplotypes, the ACC haplotype was associated with Gram-negative bacteremia (*p=0.04*) and superinfection (*p=0.048*). **Summary and Conclusions.** The results showed that despite the primary predisposition to infection typical of multiple myeloma, the presence of the variants studied significantly affected susceptibility to serious infections and outcomes.

0432

PROTECTIVE EFFECT OF ANTI-FUNGAL AND ANTI-BACTERIAL PROPHYLAXIS IN AML THERAPY: A SINGLE CENTER EXPERIENCE

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Background. Supportive therapy for patients with acute myeloid leukemia undergoing intensive chemotherapy has improved during the last decades. However, infections remain a major concern and the optimal strategy to reduce infection related morbidity and mortality is still a matter of debate and efficacy might be largely dependent on local settings. At our institution we did not apply prophylaxis until March 2010, when we introduced an anti-infective chemoprophylaxis scheme with posaconazole, levofloxacin and trimethroprim/sulfamethoxazole in combination with antiemetic prophylaxis with dexamethasone for all patients. **Aims.** To assess the impact of the newly introduced primary prophylaxis scheme on the incidence of fungal infections and bacterial infections, respectively. **Methods.** Retrospective single-center study performed at the leukemia ward (normal ventilation) and hematopoietic stem cell transplantation unit (air filtration and positive-pressure) of the University Hospital Zurich between 2009 and 2011. All Patients >=18 years with AML or high-risk MDS (IPSS>1.5) receiving intensive chemotherapy were included. Statistical analysis compared the patient cohorts before and after introduction of the prophylaxis scheme in March 2010. Invasive fungal infections (IFI) were classified according to the EORTC/MSG guidelines. **Results.** The study comprised 88 patients receiving a total of 203 therapy cycles. Baseline characteristics were similar in the non-prophylaxis and the prophylaxis cohort. The median age of the study population was 51.3 years (IQR 20.7), 45 (51%) of all patients were male. Five and 7 patients in the non-prophylaxis and prophylaxis cohort were diagnosed with IFD before the start of chemotherapy and were excluded from the analysis. Aplasia time was significantly longer in the non-prophylaxis cohort beeing 20 (IQR 10) and 17.5 (IQR 8.8) days, respectively (p=0.011). In the non-prophylaxis cohort more chemotherapy cycles were performed at the stem cell transplantation unit (29.3% and 9.6%, respectively; p=0.012). Adherence to the new prophylaxis scheme was 81.7% for posaconazole and 69.5% for levofloxacin, respectively. Overall survival at 100 days was 88.4% in the non-prophylaxis and 84.4% in the prophylaxis period, respectively. Invasive fungal infections were significantly more common in the non-prophylaxis period than in the prophylaxis period (88.9% and 55.3%; p=0.0032). In both groups IFI were classified as possible' (EORTC/MSG) in the vast majority of all cases (93.5% and 81%). In multivariate analysis chemoprophylaxis a well as treatment in the hematopoietic stem cell transplantation unit both led to a significant reduction in IFI (p<0.0001 and p=0.0021, respectively). No difference in bacteremia incidence was observed (32.6% and 34.6% in all chemotherapy cycles in the non-prophylaxis and the prophylaxis group, respectively). However, bacteremia in the prophylaxis group was more likely to be caused by gram-positive bacteria than in the non-prophylaxis-group (91.5% vs. 71.8%).Summary/ **Conclusions.** Our data confirm, that our combined antifungal and antibacterial primary prophylaxis with posaconazole and levofloxacin as well as treatment in a positive-pressure ward resulted in a significant reduction of possible IFI, however it did not reduce the incidence of bacteremia.

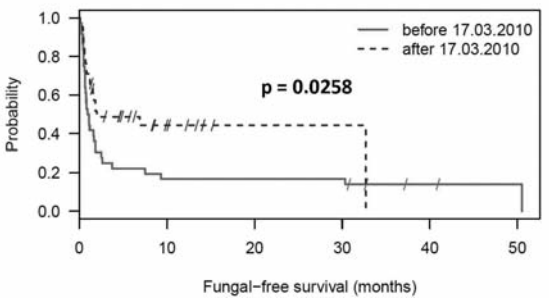

Figure 1.

0433

A COMPARATIVE STUDY OF PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA RECEIVING ALL-TRANS RETINOIC ACID WITH AND WITHOUT VORICONAZOLE: EFFECT ON DIFFERENTIATION SYNDROME

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Background. The differentiation syndrome (DS) is a potentially fatal complication related to all *trans*-retinoic acid (ATRA) use in induction chemotherapy for patients with acute promyelocytic leukemia (APL). The reported incidence and mortality rates range between 2-31% and 1-33% respectively. The metabolism of ATRA is mediated through human cytochrome P450 (CYP) enzyme pathways, most notably CYP2C9 and CYP3A4. Triazole derivatives, such as voriconazole, are potent antifungal agents that act by inhibiting the CYP enzyme mediated conversion of lanosterol to ergosterol. Voriconazole is known to be a strong inhibitor of CYP2C9 and CYP2C19 in contrast to fluconazole which has less potent CYP interactions. **Aims.** With the increasing use of voriconazole for fungal prophylaxis, we wished to evaluate and contrast the inci-

dence and outcomes of ATRA-induced DS in patients with APL. **Methods.** Forty-six consecutive patients with APL undergoing induction-phase chemotherapy utilizing ATRA were seen at Mayo Clinic from 2000 through 2011. All patients provided consent for research participation. All patients underwent bone marrow examination with cytogenetic and molecular evaluation including FISH and RT-PCR for *PML-RARA*. DS was defined in accordance with other studies. Comparisons between the groups for categorical outcomes were made using Pearson's chi-square or Fisher's exact test. Two-sample t-tests or Wilcoxon rank-sum tests were used for continuous outcomes. A Cox proportional hazards model measured the association of voriconazole use and body mass index (BMI) with DS occurrence, where voriconazole use was considered a time-dependent covariate. **Results.** Of the 46 study patients, 27 (58%) were male with a median age of 56 years (range, 18-80 years). Thirty-one patients (67%) received a chemotherapy regimen including ATRA coinciding with voriconazole administration and 15 patients underwent chemotherapy treatment including ATRA without fungal prophylaxis. There was no difference in age, gender, Sanz risk assessment, combination chemotherapy regimen, white blood cell count, platelet count, creatinine clearance and LDH levels amongst patients in the two groups. The only heterogeneity was with the BMI, which was higher in patients receiving voriconazole (HR 1.04, CI 1.001-1.078, p=0.0427). The overall incidence of DS was 35% (n=16), with patients receiving voriconazole being more likely to experience the same (HR 2.31, CI 0.78-6.874, p=0.1308). After adjusting for BMI, patients receiving voriconazole had a higher tendency to experience DS (especially severe DS; 13 of 16 cases [81%]), however due to small numbers this trend was not statistically significant (HR 1.96, CI 0.65-5.94, p=0.23). Seven patients (44%), 5 of whom had received voriconazole, needed ICU admission for management of severe DS. Mean length of ICU stay was 4 days (range, 1-7 days) with no patients requiring intubation but 29% receiving vasopressor support. There were zero deaths attributable to DS and the Kaplan-Meier curve provided estimates days from initiation of voriconazole to diagnosis of DS. **Conclusions.** A trend towards increased incidence and severity of the ATRA mediated DS was seen in APL patients receiving voriconazole as fungal prophylaxis during induction therapy, contributing to the morbidity. Statistical significance was not reached due to small sample size; however this finding warrants larger studies addressing this important issue.

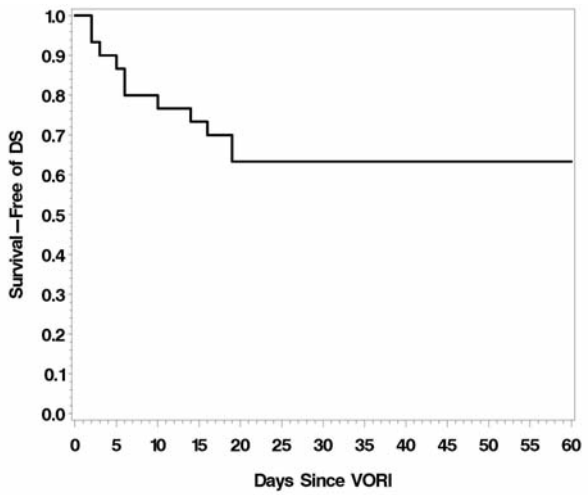

Figure 1.

0434

EARLY DIAGNOSIS OF INVASIVE FUNGAL DISEASE IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES. PROSPECTIVE EVALUATION OF BIOLOGICAL MARKERS

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Background. Invasive fungal disease (IFD) is an important cause of morbidity and mortality in patients with hematologic malignancies. Last years, the detection of biomarkers has become indispensable for the early diagnosis of fungal infections in these patients.OBJECTIVE. We evaluated the accuracy of serum galactomannan antigen (GM), (1-3) β-D-glucan antigen (BG) and antibodies against *Candida albicans* germ tubes (CAGT) for early diagnosis of IFD in patients with hematologic malignancies. **Methods.** A total of 500 serum samples from forty five hematology patients at high or intermediate-high risk for IFD as defined by Prentice et al. were collected twice weekly, from January 2008 through December 2010 ina single center. Quantitative detection of GM (Platelia *Aspergillus*®; Bio-Rad), BG (Glucatell® test) and antibodies to CAGT (*C. albicans* IFA IgG Vircell®test) were performed in every sample as surveillance test. In the presence of signs or symptoms suggestive of IFD or in case of positive test, patients were assessed and diagnosed according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycosis Study Group (EORTC/MSG) Consensus Group.RESULTS. Seventy two episodes were assessed in the entire group. Median age of patients was 62 years (range 31-80). The most frequent underlying disease was acute myeloid leukemia (n=31). Four proven IFD and eight probable IFD were diagnosed in ten patients (22,2%). *Aspergillus* spp. was isolated in two patients, *Candida* spp in two patients and *Saccharomyces cerevisiae* in one patient. Pneumonia was the most common infection, ten of twelve IFD diagnosed.Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of GM monitoring at a cutoff of 0.5 were 100% (CI 95%: 93.7-100%), 57.5% (CI 95%: 40-74%), 32% (CI 95%: 11.7-52.2%), 100% (CI 95%: 97.5-100%) respectively, with an area under the curve (AUC) of 0.841. The sensitivity, specificity, PPV and NPV of BG monitoring at a cutoff of 120 pg/ml were 44.4% (CI 95%: 6.42-82.46), 57.5% (CI 95%: 39.2-75.9%), 22.2% (CI 95%: 0.24-44.2%), 80% (CI 95%: 60.8-97.5%) respectively, with an AUC of 0.6128. The diagnostic value of detection of antibodies to CAGT was not analyzed due to the low incidence of invasive candidiasis (two cases). False-positive results from GM and BG test occurred in 26.6% and 25% cases respectively.Of note, the combined results of GM and BG resulted in an improvement in the diagnostic accuracy of the tests to predict IFD, with sensitivity 57.5%, specificity 88.2%, PPV 43% and NPV 85.7%. **Conclusions.** The prospective screening of GM, BG and antibodies to CAGT is a useful and non-invasive tool for early diagnosis of IFD in high or intermediate-high risk adult hematologic patients. The combined use of GM and BG detection results in a more accurate strategy compared to the single determination of any of them, by increasing the specificity and the PPV whereas keeping a high NPV.

0435

A MULTICENTER, PROSPECTIVE STUDY FOR INVASIVE FUNGAL DISEASE IN CHINESE PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background. The morbidity and mortality of invasive fungal diseases (IFDs) in patients with hematological malignancies undergoing chemotherapy remain high despite of new early diagnostic methods and antifungal prophylaxis. **Aims.** To better understand the epidemiology, diagnosis and treatments practice of IFDs in patients with hematological malignancies in China. **Methods.** This is the first multicenter, prospective observational study conducted in hematological centers in China. Patients admitted on the 1st to 10th day of each month from January to August 2011 and received intravenous chemotherapy for treatment of leukemia, hodgkins lymphoma, non-hodgkins lymphoma (NHL), myelodysplastic syndrome, and multiple myeloma were enrolled from 35 hospitals. Patients were followed through discharge and to the next course of chemotherapy. Underlying disease, previous IFD, medical history, antifungal prophylaxis and treatment during hospitalization were collected for all enrolled patients regardless of antifungal therapy. Patients diagnosed as proven or probable IFDs were followed up for an additional 6 weeks. **Results.** A total of 4816 patients were enrolled. Patient's age ranged from 1 to 90 yrs with median of 43 yrs, males accounted for 59%. Acute myeloid leukemia (AML) (27.8%), NHL (27.6%) and Acute lymphocytic leukemia (ALL) (19.7%) were the major underlying hematological diseases. Twenty percent of patients received first induction chemotherapy. One-third of the patients had ANC<500/mm^3, and 5.5% of the patients had previous invasive fungal infection.During hospitalization 18.8% of the p atients received antifungal prophylaxis, and the most commonly used agents for prophylaxis were azoles (94%). Among patients received antifungal prophylaxis, one-fifth received therapeutic antifungal treatment later. A total of 609 (12.6%) patients received therapeutic antifungal treatment, among them empirical therapy (88%) is the most common strategy when initiating antifungal therapy, pre-emptive and definitive therapy accounted for 8% and 4%, respectively. The most common agents for initiating treatment were azoles (80.8%), polyenes and echinocandins accounted for 10.2% and 11.8% respectively. After initial antifungal treatment 33.8% patients has adjusted treatment protocol, mainly because of lack of effectiveness (50.5%). The median duration of antifungal treatment was 14 days.A total of 8.6% of the patients were diagnosed as IFD, 172 (3.6%) patients were diagnosed as proven or probable IFD. The top three underlying diseases with high morbidity of IFD (proven or

probable) were AML (7.1%), MDS (4.1%) and ALL (3.7%). Univariate analysis showed that the previous IFDs, lower ANC, higher ECOG score, renal dysfunction and low albumin were associated with the development of IFDs.The overall mortality is 1.4%, while the mortality in patients with proven or probable IFD was 11.1%. **Conclusion.** The incidence of IFDs is relatively high in patients with hematological malignancies undergoing chemotherapy in China, which was similar to findings from Europe and U.S. The mortality rate in IFD is significantly higher than overall patients. The proportion of empirical antifungal therapy is higher in China which could be due to difficulties of early diagnosis of IFDs.

0436

THE DETECTION OF 1,3-?-D-GLUCAN DETECTION IN BRONCHOALVEOLAR LAVAGE FLUID FOR THE DIAGNOSIS OF INVASIVE PULMONARY ASPERGILLOSIS IN HEMATOLOGICAL PATIENTS

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Background. Invasive pulmonary aspergillosis (IPA) is a severe infectious complication of hematological patients and its early diagnosis can contribute to the improvement of survival of affected patients. **Aims.** To evaluate the contribution of 1,3-β-D-glucan (BG) performed from bronchoalveolar lavage (BAL) fluid to IPA diagnosis. **Methods.** A retrospective analysis of BG detection in BAL fluid performed in 163 hematological patients between 1/2007 and 12/2011. Proven IPA (EORTC/MSG 2008 criteria) was documented in 2.6% (5 pts.), probable IPA in 10.7% (20 pts.) and possible IPA in 40.0% (75 pts). 46.7% (87 pts.) without evidence of IPA were considered as negative control. Pneumocystis pneumonia was diagnosed in 7.0% (13 cases) and positive culture from BAL fluid for *Candida sp*. was in 4.8 % (9 cases). Oral cavity colonization with *Candida sp*. was detected in 33.7% (63 cases). **Results.** 187 samples were included in the analysis. 48% (12/25 pts) of samples from proven/probable IPA pts were positive (> 80 pg/ml) and 12% (3/25) were intermediate (60-80 pg/ml). 37% (32/87) samples from pts without IPA were positive and 2% (2/87) were intermediate. After the exclusion of samples with pneumocystis pneumonia, the number of positive and intermediate results decreased to 32%.The mean BAL BG for patients with proven and probable IPA was 79 pg/ml (range 0-1145), for possible IPA 13 pg/ml (range 0-534) and for negative control 55 pg/ml (range 0-1477).The frequency of false positive results (>80 pg/ml) in our study was 29%. There was no difference between the mean BAL BG in the control group with or without oral cavity colonization with *Candida sp*. (43 vs. 32 pg/ml respective).The performance of glucan in BAL fluid tested at various cutoffs is presented in the table. **Summary and Conclusions.** The performance of glucan in BAL fluid has acceptable sensitivity, but a low specificity and positive predictive value for the diagnosis of invasive pulmonary aspergillosis. Moreover, due to "panfungality"of the assay it is necessary to use a combination of methods (ie galactomannan or PCR) for the confirmation of IPA.Supported by Masaryk University MUNI/A/0784/2011.

Table 1. The performance of glucan in BAL fluid tested at various cutoffs.

Proven and probable IPA vs. control group without pneumocystis pneumonia		
Sensitivity	88.0% (67.6 - 96.8%)	76.0% (54.4 - 89.9%)
Specificity	68.9 (56.9 - 78.9%)	70.2% (58.8 - 80.0%)
PPV	48.9% (33.9 - 64.0%)	46.3% (30.9 - 62.3%)
NPV	94.4 (83.6 - 98.5%)	89.6% (78.1 - 95.7%)

0437

BRAIN ABSCESS AND FULMINANT SEPSIS CAUSED BY BACILLUS CEREUS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES: CLINICAL EPIDEMIC FEATURES AND OUTCOMES

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Background. Patients with haematological malignancies develop several kinds of bacteraemia due to intensive cytotoxic chemotherapy and immunosuppressive treatments. *Bacillus cereus* has emerged as a gram-positive pathogen that causes serious infection in haematological patients. However, the risk factors and characteristics have not been fully elucidated. **Patients and Methods.** We performed a retrospective chart review of the clinical and epidemiological feature of *Bacillus* bacteraemia in haematological and non-haematological patients at our hospital. In patients with sepsis, we also reviewed their charts to obtain clinical information, including insertion of a central venous catheter, neutrophil count, prior chemotherapy or steroid treatment.We also investigated clinical signs at febrile events, such as CNS involvement, unstable vital signs, gastrointestinal symptoms, antibiotic use and drug sensitivity of *Bacillus* species.We used univariate analysis and assessed the risk factors for fatal prognosis and brain abscess by *B. cereus*. Statistical analysis included χ^2 and Fisher's exact tests. All calculation were performed using the program R 2.12.1. **Results.** At our institution, we identified 56 patients with positive blood cultures for *B. cereus*, 17 with and 39 without haematological disease, during the period from April 2004 through December 2011. Bacteraemia was defined by the isolation of *B. cereus* from more than two blood culture sets. If a single blood culture yielded *B. cereus* without clinical symptoms, it was considered due to contamination. With respect to underlying haematological malignancies, 9 patients had leukaemia, 4 had myeloma, 3 had lymphoma and one had other haematological malignancies. Meningitis and brain abscess occurred in 3 of the 17 patients with haematological malignancies, but in none of those without haematological malignancies. Similarly, we noted that 5 of 17 patients with and 4 of 39 patients without haematological malignancy showed unstable vital signs caused by septic shock. Of the 17 patients with haematological malignancies, 3 patients died of *B. cereus* sepsis within few days, and 1 patient died of *B. cereus* meningitis and brain abscess despite receiving appropriate antibiotic therapy. In contrast, only 1 of the 39 patients without haematological malignancies died of *B. cereus* sepsis. Potential risk factors for brain abscess development in patients with *Bacillus* septicaemia included neutrophil count < 500/mm^3, corticosteroid use within 14 days and haematological malignancies. (P=0.003, P=0.007, P=0.003, respectively). Compared with the patients without malignancies, *B. cereus* septicaemia in patients with haematological malignancies was significantly associated with fatal outcome (P = 0.03). **Conclusions.** This study indicated that *B. cereus* causes fulminant meningitis, brain abscess and fatal prognosis in patients with, but not in those patients without, haematological malignancies. Therefore, early therapeutic intervention against *B. cereus* sepsis is important in the treatment of haematological malignancies.

0438

PNEUMOCYSTIS JIROVECI PNEUMONIA IN HEMATOLOGICAL PATIENTS: A SINGLE INSTITUTION EXPERIENCE

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Background. Pneumocystis jiroveci pneumonia (PJP) is usually documented in HIV infection and, in general, in patients with compromised immune system. It has been reported an incidence of PJP infection in hematological malignancies higher than in solid tumors. **Aims.** To establish the frequency and clinic characteristics of PJP infection in consecutive patients with hematologic neoplasms over a period of 2 years **Methods.** Patients population comprised consecutive HIV negative adult patients with hematological cancer and clinically suspected of PJP during the period 2008-2010. The diagnosis was done using PCR for PJ performed on oral wash. All the cases with microbiologically documented PJP were analyzed considering the following variables: demographic data, underlying hematological malignancy, relationship between chemotherapy and diagnosis of PJP, clinical parameters and radiological reports related to pneumonia and lymphocyte count. We obtained informed consent for samples taking and related analyses. **Results.** Among 86 consecutive subjects with hematologic malignancies, 24 resulted affected by PJP (27.9%). Mean age was 59±18.8 years; 14/24 (58.3%) of them were male and 10/24 (48.7%) female. The underlying hematologic diseases were: Non-Hodgkin lymphoma (66.7%), Hodgkin lymphoma (20.8%), Acute Myeloid Leukemia (8.3%) and aplastic anemia (4.2%). All the patients received chemotherapy (as single therapy or in combination); the most common regimens were FCR, CHOP and ABVD. No one received bone marrow transplantation at the time of present analysis. Diagnosis of PJP infection was established before chemotherapy in 14.5%, during treatment in 37.5% and after chemotherapy in the remaining 48% of the patients. Trimethoprim/sulfamethoxazole (TMP-SMZ) prophylaxis was performed in 5 PJP patients only. Mean value of lymphocyte count was 0.86 x 10^9/L (r. 0.10 -2.36 x 10^9/L; ±0.615). At the time of diagnosis, a statistical difference in median lymphocyte count was observed between patients < or > 65 years old [median count: 0.60 (± 0.42) x 10^9/L vs 1.13 (L ± 0.68) x10^9/, p < 0.005]. After a mean of 27.6 days (± 30.9), oral wash test became negative in cases assessed after PJ therapy. None of patients died or had serious adverse events. **Conclusions.** The incidence and clinical characteristics of PJP in hematological patients receiving chemotherapy is still under investigation; we found a broad spectrum of hematologic diseases complicated by PJP. In our population, diagnosis of PJ infection was established in > 10% of patients before the beginning of the first cycle of chemotherapy. Because of non-invasive oral washes PCR examination, appropriate designed studies should address the clinical advantages of the early diagnosis of PJ infection (and its treatment before chemotherapy) in heamatological malignancies.

Stem cell transplantation - Clinical 1

0439

CO-MORBIDITY SCORING IS A CRITICAL DETERMINANT OF OUTCOME AFTER ALEMTUZUMAB BASED REDUCED INTENSITY ALLOGRAFTS IN PATIENTS OVER THE AGE OF 60: A BSBMT STUDY

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Introduction. Reduced intensity allogeneic transplants represent a curative therapy in older patients with haematological malignancies. However acute and chronic graft-versus-host disease (GVHD) remain major complications, particularly in older patients, and there is increasing use of *in vivo* T cell depletion using alemtuzumab or ATG in this patient population. Such a manoeuvre is associated with delayed immune reconstitution and increased infectious complications but there has been no analysis of factors predicting survival after a T cell depleted allograft in older patients. **Aims.** We therefore wished to identify factors determining overall survival (OS) and non-relapse mortality (NRM) after an Alemtuzumab based RIC allograft in patients with a haematological malignancy over the age of 60. In addition to standard transplant criteria the Seattle Co-morbidity score was applied in order to study the impact of pre-transplant co-morbidity on patient outcome. **Patients and Methods.** 215 patients with a haematological malignancy underwent allogeneic transplantation using an alemtuzumab based RIC regimen. The median patient age was 63 years (range 60-73). 134 patients had a Seattle Co-morbidity score of 0 or 1 and 57 a score of 2 or more (data unavailable in 24). 165 patients underwent transplantation for a myeloid malignancy (AML n=92, MDS n=60, miscellaneous n=11) and 50 for lymphoid malignancies (CLL n=20, NHL n=15, miscellaneous n= 15). 138 patients underwent a transplant from a matched (7/8 or 8/8) unrelated donor and 77 from a matched sibling donor. All patients were transplanted using alemtuzumab based conditioning (Fludarabine/Melphalan/alemtuzumab n=141, miscellaneous (including BEAM alemtuzumab and busulphan/alemtuzumab n=74). The median follow up was 24 months (range 2-100). **Results.** The one year and five year overall survival was 56% (49-63%) and 36% (27-45%) respectively. The one year non relapse mortality (NRM) was 27% (21-34%). The one year and 5 year relapse rates were 23% (17-29%) and 39% (30-48%) respectively. 25 patients (11%) developed Grade III-IV acute GvHD and 16 (7%) patients developed chronic extensive GvHD. OS was influenced by status at transplant (CR/stable disease 47% v 31% at 2 years p=0.045) and co-morbidity score (score 0/1 59% at 2 years versus 7% for higher score p=0.0001). In multivariate analysis NRM was influenced by co-morbidity score (p=0.001) and a dose of CD34+ cells >5.5x10^6 CD34/kg (p=0.028). OS was influenced by disease status at transplant (p=0.003) and co-morbidity score (p=0.001) but not patient age. **Conclusions.** Alemtuzumab based RIC allografts can be delivered safely in patients above the age of 60. The most important determinants of outcome are disease status at transplant and co-morbidity score. This is the first study to examine the impact of patient co-morbidities on outcome after a T cell depleted RIC allograft. Whilst our data require confirmation in a larger cohort of patients they suggest that meticulous co-morbidity scoring is mandatory in older patients who can benefit from a RIC allograft

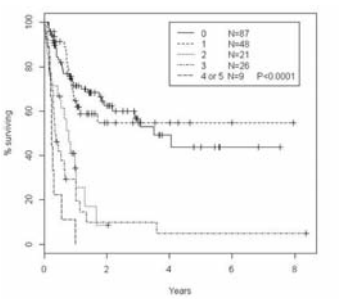

Figure 1. Overall survival by seattle comorbidiy score.

0440

FLUDARABINE AND TREOSULFAN CONDITIONING FOR ALLOGENEIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH AML AND MDS NOT ELIGIBLE FOR STANDARD MYELOABLATIVE CONDITIONING

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Background. Allogeneic stem-cell transplantation (SCT) is curative approach in AML and MDS. However, standard myeloablative conditioning (MAC) is limited to younger, medically fit patients. Reduced-intensity conditioning (RIC) is feasible in patients not eligible for MAC, however relapse rates are increased, especially in patients not in remission at SCT. Novel approaches are needed to deliver dose-intense conditioning, yet with RIC type toxicity, to these patients. Treosulfan is a bifunctional alkylating agent with cytotoxic and immunosuppressive effects. Prior studies demonstrated the feasibility of fludarabine-treosulfan(FT) conditioning, suggesting it is effective with limited toxicity.*Aims* & **Methods.** To better define the role of FT, we analyzed outcomes of a relatively large cohort of 202 patients with AML (n=128) or MDS (n=74) treated in two institutions. Patients were eligible for FT only if considered non-eligible for MAC. FT included fludarabine 30 mg/m^2 x5 days and treosulfan 12 gr/m^2 x3 days in one institution (FT12, n=109) or 14 gr/m^2 x3 in the second institution (FT14, n=93). **Results.** The median age was 58 years (21-76). The donor was an HLA-matched sibling (n=96), matched unrelated (n=96) or alternative donor (n=10); 35 patients (17%) had an HLA-mismatched donor. Disease status was CR1 (n=62), CR2/later CR (n=31), no CR (n=40) or previously untreated MDS (n=69). 55 patients had comorbidity score>2 (27%). 194 patients engrafted in a median of 15 days (9-28), 8 died prior to engraftment (4%) and 3 had primary non-engraftment (1.5%). With median follow-up of 34 months (1-93), 100 patients are alive and 102 have died. The cumulative incidence of acute GVHD grade II-IV and III-IV was 30% and 20%, respectively. The cumulative incidence of chronic GVHD was 41%. The 3-year non-relapse (NRM) and relapse mortality was 28% (95%CI, 22-36) and 27% (95%CI,21-35), respectively. The 3-year overall survival rate (OS) was 45% (95%CI, 37-52). OS was 63% (95%CI, 50-76), 39%(95%CI, 20-58), 20% (95%CI, 7-33) and 46% (95%CI, 32-60) in patients in CR1, CR2/later CR, no CR or previously untreated, respectively (p<0.001). Disease status at SCT was the only prognostic factor in multivariate analysis. Patients not in CR had hazard-ratio of 4.2 (p<0.001) for shortened OS, 4.9 (p=0.001) for relapse, and 2.6 (p=0.04) for NRM. There was no significant difference in any of these outcomes between FT12 and FT14. However, there was a trend for better OS with FT12 compared to FT14 in patients in CR1, 85% Vs. 56%, respectively (p=0.1) due to lower NRM in this subgroup. Among intermediate-risk patients (CR2/ previously untreated) OS was 40% and 49%, respectively, due to less relapses with FT14 in this subgroup, but not reaching statistical significance. There was no difference in the poor-risk subgroup (no CR). **Conclusions.** This relatively large cohort defines the expected outcomes with treosulfan-based conditioning. This is a promising approach in MAC- ineligible patients with outcomes that are similar to MAC. Favorable results were observed in patients in CR or untreated MDS at SCT. The role of different treosulfan doses should be further defined in prospective studies.

0441

INDUCTION THERAPY WITH BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE (VTD) DOES NOT IMPAIRE AUTOLOGOUS PERIPHERAL BLOOD STEM-CELL (PBSC) COLLECTION IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

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Background. The novel agents bortezomib, thalidomide and lenalidomide have been successfully incorporated into autologous stem-cell transplantation (ASCT) for MM. However, several concerns have been raised about the impact of novel agent-based induction therapies on PBSC collection. **Aims.** The GIMEMA Italian Myeloma Network designed a phase III study to compare VTD with thalidomide-dexamethasone (TD) as induction therapy prior to double ASCT. We compared the effect of VTD versus TD induction regimens on CD34+ cell collection. **Methods.** After three 21-day cycles of VTD or TD induction therapy, patients received cyclophosphamide (CTX, 4 g/m^2) followed by G-CSF (10 mcg/Kg/die). The target threshold to safely perform double ASCT was 4 x 10^6 CD34+ cells/Kg. **Results.** Of 474 patients who received induction therapy, 435 (223 in VTD and

212 in TD) were evaluable for PBSC collection. The median number of collected CD34+ cells was 9.7 x 10^6/Kg in VTD arm and 10.7 x 10^6/Kg in TD arm (p= 0.220). The planned yield of 4 x 10^6 CD34+ cells/Kg was achieved with a single harvest in 91% of patients in both treatment arms (p= 0.867). Collected CD34+ cells >10 x 10^6/Kg (50% in VTD and 58% in TD) and collection failure (2% each) did not differ between treatment groups (p= 0.228). Most of patients (86% in VTD and 82% in TD, p=0.320) received CTX as an in-patient procedure (median hospitalization: 4 days); less than 5% of patients developed grade 3-4 infectious complications. Following ASCT, no significant difference was observed between VTD and TD in terms of hematologic recovery and non hematological toxicity. Kaplan-Meier curves of TTP and PFS were almost superimposable for patients with a CD34+ yield in the range between 4 and 10^6/kg and >10 x 10^6/Kg or (group 1), as well as for those who collected between 2 and 4 x 10^6/Kg and <2 x 10^6/Kg (group 2). The 3-year estimates of TTP and PFS were 70% for group 1 vs 39% for group 2 (p= 0.0000) and 69% vs 35% (p= 0.0000), respectively. OS rate at 3 years was 90% in group 1 vs 69% in group 2 (p= 0.0002). In a multivariate Cox regression analysis, a yield of CD34+ cells >4 x 10^6/Kg was independently associated with prolonged PFS (p=0.000), and extended OS (p=0.000). Absence of t(4;14) and/or del(17q) and beta2-microglobulin <3.5 mg/L were favorable prognostic factors for both PFS and OS; randomization to VTD independently predicted for longer PFS. **Summary and Conclusions.** Both VTD and TD regimens did not adversely affect PBSC collection and the engraftment potential of collected PBSCs. As a result of early PBSC collection and use of CTX plus G-CSF, the target of >4 x 10^6/Kg CD34+ cells was achieved with a single harvest in more than 90% of patients, and collection failure was seen in only 2%. Notably, the collection of >4 x 10^6 CD34+ cells/Kg was independently associated with extended PFS and OS.

0442

TREOSULFAN-BASED CONDITIONING FOR CORD BLOOD TRANSPLANTATION IS SAFE AND EFFECTIVE: RESULTS FROM AN ONGOING PHASE-II STUDY

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Backgrounds. Treosulfan (is an immunosuppressive and myeloablative alkylating agent recently introduced as a conditioning agent for hematopoietic cell transplantation (HCT). While previous studies have investigated the role of treosulfan-based conditioning regimens for conventional donor HCT, the experience in cord blood transplantation (CBT) is limited. **Methods.** Between March 2009 and November 2011, 33 consecutive CBT recipients were enrolled in this prospective non-randomized Phase II study at Fred Hutchinson Cancer Research Center. The conditioning regimen consisted of Treo (14 gm/m^2/day) from day -6 to day -4, Fludarabine (30 mg/m^2/day) from day -6 to day -2, and a single fraction of 200cGy TBI on day -1. Two patients deemed at high risk of graft failure received a total dose of Flu equal to 35 mg/m^2/day. Graft versus host disease (GVHD) prophylaxis consisted of cyclosporine and mycophenolate mofetil. All CBT grafts were 4-6/6 HLA-matched (A and B antigen level, DRB1 allele level) to the patients. Engraftment, non-relapse mortality (NRM), relapse and GVHD were calculated using cumulative incidence rates to accommodate competing risks. Disease-free survival (DFS) and overall survival (OS) were analyzed using Kaplan-Meier estimates. **Results.** All but 2 patients received a double CBT, with a median dose of total nucleated and CD34+ cells of 3.6 x 10^7/kg (range, 2.2-10.2) and 0.19 x 10^6/kg (range, 0.04-0.66), respectively. The median age was 52 years (range, 5-63) and weight 76 kg (range, 17-114). The median follow-up of the survivors was 300 days (range, 105-1085). Disease status pre-transplant included acute myeloid leukemia in 1st complete remission (CR1) (n=9) and in CR2 (n=5), acute lymphoblastic leukemia in CR1 (n=3) and in CR2 (n=8), myelodysplastic syndrome (n=6) and myeloproliferative disorder (n=2). Fifteen patients (47%) had minimal residual disease at the time of transplant. Five patients (15%) had failed a prior myeloablative allogeneic HCT. Median time of neutrophil recovery was 23 days (range, 13-89 days) with a cumulative incidence of engraftment of 93% (95% CI, 76-98%). Two patients (6%) experienced primary graft failure. The median time to platelet recovery (>20 x 10^9/L) was 34 days (range, 24 - 62) with a cumulative incidence at day 100 of 73% (CI 95%: 56 - 81). The probability of 2-year OS and DFS was 66% (95% CI, 44-81%) and 60% (95% CI, 37-78), respectively. The cumulative incidence of NRM at 2 years was 22% (95% CI, 8-40%) and relapse 17% (5-36%). Ten (30%) patients died, with relapse/multiorgan failure as the most common causes (n=7). The cumulative incidence of grade II-IV acute GVHD was 66% (95% CI, 47-80) and grade III-IV 23% (95% CI, 10-39%), whereas chronic GVHD at 1 year was 28% (95% CI, 10-45%). **Conclusions.** The preliminary results presented herein from this first prospective CBT trial utilizing treosulfan-based conditioning demonstrates that this approach is well-tolerated and sufficiently immunosuppressive to promote sustained CB donor engraftment. Importantly, this regimen extends donor options for patients in need of myeloablative HCT without conventional donors who are ineligible for other myeloablative regimens, especially those containing high dose TBI. A large randomized study is now needed to confirm these favorable outcome results.

0443

THE PROGNOSTIC SIGNIFICANCE OF PET SCAN BEFORE AND AFTER HIGH-DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA (HL) PATIENTS

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Background. ASCT is the standard approach for relapsed/refractory HL patients. Chemosensitivity prior to ASCT is a powerful prognostic factor for outcome; the predictive significance of PET scan in the ASCT setting has not been firmly established yet. **Aims.** To further update our previous results (presented in EBMT 2010) with respect to the prognostic significance of PET scan before and after HDT/ASCT in a series of 68 patients with relapsed/refractory HL from a single Transplant Unit. **Methods.** Computed tomography and PET scan were performed just prior to ASCT and at 3 months post transplant. Findings were correlated with failure free survival (FFS). PET scan was considered negative, when no uptake was present, positive, when any lesion was FDG-avid with SUV ≥4 and minimal residual uptake positive (MRUp), when any lesion showed abnormal uptake with SUV<4. MRUp cases were analyzed as positive pre-ASCT and as negative post-ASCT. Patients achieving a complete or partial remission prior to ASCT as assessed by conventional methods were considered chemosensitive.

Table 1. Relapses according to pre- and post ASCT PET scan, N=53.

Pre-ASCT PET-scan	Post-ASCT PET-scan	Relapses/patients	2-year FFS (%)	p
negative →	negative	2/16	86±7	P<0.0001
positive →	negative	1/17	93±7	
negative →	positive	1/2	50±35	
positive →	positive	15/16	6±6	

Results. Among 68 patients included in the study, 47% were treated for primary refractory disease, 41% at first relapse and 12% beyond first relapse. All patients received salvage chemotherapy and 75% had chemosensitive disease prior to ASCT. A PET-scan was available pre-ASCT in 55 patients, post ASCT in 62 and at both time points in 53. Pre-ASCT PET scan was positive or MRUp in 24/45 chemosensitive patients vs 10/10 chemoresistant ones (p=0.02). There was no chemoresistant patient with a negative PET-scan. Pre-ASCT PET-scan was of prognostic significance for outcome: 3/21 patients with a negative pre-ASCT scan relapsed vs 17/34 with a positive or MRUp one, resulting in a 2-year FFS of 85% and 48% respectively (p=0.005), Thus almost

50% of pre-ASCT PET(+) patients did not relapse at a median follow-up of 30 months (4-93), while a plateau was reached at 15 months post-transplant. Chemosensitivity remained significant as expected: 2-year FFS was 71% and 24% for chemosensitive and chemoresistant patients respectively (p=0.0001) Chemosensitivity could further stratify pre-ASCT PET(+) patients: Chemosensitive PET(-) patients had a 2-year FFS of 85%, vs 56% for chemosensitive PET(+) and 30% for chemoresistant PET(+) ones (p=0.002). Post-ASCT PET scan had the strongest predictive value for outcome: 2-y FFS was 87% for post ASCT PET(-) or MRUp cases vs 19% for PET(+) ones (p< 0.0001). When we further compared the results for the 53 patients who had a PET-scan available both pre- and post-ASCT, we found that patients who became PET(-) post ASCT had an excellent outcome irrespectively of pre-ASCT PET-scan findings, whereas almost all PET(+) ones relapsed, as shown in the Table 1. **Conclusions.** Pre-ASCT PET scan positivity does not preclude a favorable outcome for patients with relapsed/refractory HL undergoing HDT/ASCT, since half of them remain relapse free. Chemosensitivity prior to ASCT can further discriminate the outcome of PET(+) patients. Patients who become PET(-) after ASCT have an excellent outcome in contrast to PET(+) ones whose prognosis is dismal.

0444

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA OF AMBIGUOUS LINEAGE IN ADULTS: THE COMPARISON BETWEEN STANDARD CONDITIONING AND INTENSIFIED CONDITIONING REGIMENS

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Background. Acute leukemia of ambiguous lineage (ALAL) are a rare disorders. Knowledge concerning the clinical and biological characteristics of ALAL is limited. Until now, there has been a lack of uniformity in the optimal therapeutic strategy to these cases. **Aims.** To evaluate the efficacy of two intension conditioning regimens for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in adults with ALAL. **Methods.** Fifty-three adult ALAL were enrolled in the study. Twenty-four patients received the standard conditioning (Total body irradiation (TBI) + cyclophosphamide (CY) or busulfan + CY protocol) and 29 the intensified conditioning (TBI + CY+ etoposide or fludarabine, cytarabine and TBI + CY + etoposide protocol). Thirty-four patients received related donor and 19 received unrelated donor transplantation. Cyclosporine A (CsA) was withdrawn rapidly in a stepwise fashion to avoid overwhelming Graft-versus-host disease (GVHD) reactions if acute GVHD did not develop at day +30 post-transplantation. **Results.** Of the 53 patients, 18, 19 and 16 cases received the initial induction chemotherapy protocol for acute lymphoid leukemia (ALL), acute myeloid leukemia (AML), and both lymphoid and myeloid lineages, respectively. The rate of complete remission (CR) was 83.3%, 47.4% and 75.0%, respectively, in the three protocols (*P*=0.049). All patients obtained hematopoietic reconstitution after transplantation. 3-year transplant-related mortality was 18.3±10.0% and 32.6±10.0%, respectively, in the standard conditioning and intensified conditioning groups (*P*=0.224). 3-year overall survival (OS) was 17.1±8.7% and 60.9±10.0%, and disease-free survival (DFS) was 16.2±8.2% and 57.8±10.9%, respectively, in the standard conditioning and the intensified conditioning groups (*P*=0.049 and *P*=0.009, respectively). The 3-year cumulative rate of relapse was 84.4±9.2% and 23.3±11.2%, respectively, in the standard conditioning and the intensified conditioning groups (*P*=0.000). Both univariate and multivariate analysis indicated that the patients with CR at the time of transplantation, the intensified conditioning regimen and acute GVHD all were the favorable factors to reduce the relapse. The patients with CR at the time of transplantation (*P*= 0.002 for OS; *P*= 0.011 for DFS), the intensified conditioning regimen (*P*=0.000 for OS; *P*= 0.002 for DFS) were also favorable factors to elevate the survival. **Conclusions.** CR rate of ALAL patients received initial induction chemotherapies for ALL or both myeloid and lymphoid lineages was higher than those received AML- based treatment. Intensified conditioning regimens followed by allo-HSCT might improve long-term survival and decrease relapse of leukemia in adult ALAL compared to the standard conditioning regimens.

0445

UPDATED EXPERIENCE WITH INOLIMOMAB AS TREATMENT FOR CORTICOSTEROID-REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE

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Background. Acute graft versus host disease (aGVHD) is the most important cause of non-relapse mortality after allogeneic stem cell transplantation. Response to first-line steroid therapy is obtained in 50% of the patients. Multiple second line options have been investigated with initial encouraging results; however, until now the best option, if any, in terms of improved survival remains unclear. In 2006, our group published a series of 40 patients who received inolimomab as salvage therapy for refractory aGVHD.[1] Overall response rate was 58% (CR 38%), leading to an OS of 30% at 1 year (59% for responders). Given these results, inolimomab was the standard second line treatment for aGVHD in our center. **Aims.** To analyze whether the prior promising experience was reproduced in a much larger group of patients with prolonged follow-up treated at our institution. **Methods.** We retrospectively analyzed the clinical data of 92 consecutive patients who received inolimomab as salvage treatment for refractory aGVHD between April 1999 and December 2011. All patients gave their informed consent, and the drug was administered as compassionate use. Twenty-seven (29%) had received myeloablative conditioning and 65 (71%) reduced intensity preparative regimen. Thirty-three (36%) patients had been transplanted for early disease and 59 (64%) for intermediate or advanced stage hematologic disorders. **Results.** There were 65 (71%) men. Median age was 50 years (range: 17-68). Acute GVHD developed at a median of 34 days (range: 5-170) after transplantation. Ninety-four percent of the patients presented an overall aGVHD grade III or IV at the time of starting inolimomab. The drug was initiated at a median of 17 days (range: 2-204) after diagnosis. Forty-six patients (50%) had received a prior second line immunosuppression without response. Median follow-up for survivors was 1307 days (95-4121).Overall response rate was 42% (CR 14%) on day 28. Predictors of day-28 failure to respond were: EMBT risk score >4, grade III-IV gastrointestinal involvement, female-to-male sex mismatch and lymphocyte count <$0.3x10^9$/L at starting inolimomab (p<0.01). Despite the acceptable initial response rate, results in terms of mid and long-term survival were unfavourable; during the follow-up period 79 patients (86%) died after a median of 34 (0-3068) days and 13 remained alive, for a 3-year OS of 13.2% (95% CI 5.6-20.8%), being 7% (95% CI 0-14%) and 24% (95% CI 10-38%) for day-28 non-responders and responders respectively (P<0.001). Acute GVHD was the main cause of death (42%) followed by infectious complications (26%). **Commentaries.** Our results in this update are worse than in the former report[1], highlighting the need of reporting long-term outcomes in GVHD studies. The use of inolimomab, which seems an active drug with 42% of short-term response, is associated with prolonged OS only in one fourth of responding patients. This reflects that investigating new rescue treatments with sustained effect is mandatory.

Reference

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0446

ROLE OF DONOR SPECIFIC ANTI-HLA ANTIBODIES ON GRAFT FAILURE ON CORD BLOOD TRANSPLANTATION AFTER REDUCED INTENSITY CONDITIONING REGIMEN. AN EUROCORD, SFGM-TC AND SFHI ANALYSIS

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Backgound. Delayed hematopoietic recovery and graft failure (GF) are critical complications of cord blood transplantation (CBT) and are associated with TNC or CD34 cell dose and HLA disparities. Other factors such as patients's HLA-antibodies (Ab) may impact neutrophil recovery. **Methods.** To analyze the effect of anti-HLA-Ab on CBT outcomes we analyzed 206 patients (pts) who underwent CBT after reduced intensity conditioning (RIC) from 2000-2010, in France. Median follow-up was 36 months. Eighty-two percent of pts were transplanted for malignancies, median age was 43 years. Graft composition: 82 pts (40%)

received a single CBT and 124 (60%) had a double CBT. Thirty percent of CBU had 0-1 HLA mismatch (A, B, DRB1), 67% received CyFluTBI2Gy and median infused TNC was $3.7x10^7$/Kg. Pre-transplant serum was tested for HLA-Ab with a panel of fluorescent beads coated with single HLA-antigen using Luminex™ platform. Results were interpreted as fluorescence intensity (MFI) against donor-specific mismatch (>1000 MFI was the threshold for positivity). Overall 48 pts (23%) had anti-HLA-Ab before CBT and those were donor specific anti-HLA-Ab (DSA) in 16 pts. Among the 16 pts with DSA (11 females, 5 males), 9 had single and 7 double-CBT (none had DSA directed to both CB units). Seven pts had DSA vs to HLA-Class-I, 5 vs to HLA-Class-II and 4 to both HLA-Class-I and-II. DSA threshold ranged from 1620-17629 MFI. **Results.** Cumulative incidence (CI) of day-60 neutrophil engraftment was 76%. It was 44% for recipients with DSA and 81% in pts without DSA (p=0.006). There was no difference for pts with anti-HLA-Ab non donor-specific (77% vs 69%). Multivariate model showed DSA (RR 2.7, p=0.01) and CBT before 2008 (RR 1.49, p=0.03) independently associated with GF.Seven pts with DSA engrafted, 4 after double CBT and chimerism analysis showed the engraftment of the CBU with DSA in 1 case.Among 50 pts who failed engraftment,· 9 (20%) pts had DSA specific for donor HLA-Class-I(n=4) or Class-II(n=2) or both Class-I and Class-II (n=3).CI of platelet recovery at day-180 was 62%, 12 of 16 patients with DSA did not achieve platelet recovery. CI of 1-year TRM was 35%. DSA was associated with higher TRM (p=0.002). Overall survival at 3-years was 44%, it was 41% and 45% for pts with non-malignant and malignant disease respectively. OS was 47% for recipients without DSA and 25% for those with DSA, p=0.006. In multivariate analysis, the absence of DSA was the only factor associated with better survival (RR 2.41, p=0.005). **Conclusions**. Donor-specific anti-HLA-Ab in recipients of CBT is associated with failed engraftment and lower survival. Screening for DSA may be included in the algorithm of donor choice for cord blood transplantation.

0447

ENDOTHELIAL STRESS IN STEROID-REFRACTORY GVHD: A BURDEN STATINS CANNOT LIFT

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Graft-versus-host disease (GvHD) is the major complication of allogeneic stem cell transplantation (alloSCT) and its therapy-resistant form causes significant morbidity and mortality. The pathomechanism of steroid resistance is currently not completely understood; however, we have recently suggested that endothelial dysfunction seems to play an important role (Luft et al., Blood 2011). The aim of this study was to validate in a large cohort of patients that steroid refractory acute GvHD is associated with endothelial stress. Secondly we assessed if this endothelial stress can be overcome by statins, which are known to have endothelial protective effects. **Patients and Methods.** For this retrospective study, 393 patients were eligible who had undergone alloSCT between 09/2001 and 08/2010 at our institution. Serum levels of endothelial stress markers (Angiopoietin-2: Ang-2, soluble Thrombomodulin: sTM, Interleukin-8: IL-8 and Vascular endothelial growth factor: VEGF) were compared between patients with no GvHD (n=221), grade1-2 (n=103), steroid sensitive grade 3-4 (n=27) and steroid refractory grade 3-4 (n=41) GvHD, and correlated with outcome. Serum levels of these markers were also compared between patients with and without concomitant statin treatment. **Results.** Landmark analyses at days +50 and +100 after alloSCT showed that NRM was dramatically high in the steroid refractory group but was equivalently low in the no GvHD-, sensitive grade 1-2 - and grade 3-4 - groups (p<0.0001, Figure 1). Steroid-refractory patients showed higher serum levels of Ang-2 (p=0.03) prior alloSCT and significantly stronger rises in IL-8 (day 50: p=0.006; day 100: p=0.003) and sTM (day 50: p=0.04; day 100: P=0.006) levels post alloSCT than sensitive grade 3-4 GvHD patients. High levels of sTM, IL-8, Ang-2 were significantly associated with increased NRM rates (day +50: IL8 p=0.06, sTM p=0.0008, Ang-2 p=0.0001; day +100: IL8 p=0.0007, day +100 sTM p<0.0001, Ang-2: p=0.05); even after multivariate adjustment for donor, conditioning intensity, disease status at alloSCT, and sex mismatch. In contrast, VEGF was elevated in steroid sensitive grade 3-4 GvHD patients at day 100 but did impact on NRM rates.When comparing these markers in patients who had (n=84) or had not (n=309) received concomitant therapy with statins, no difference was seen for any endothelial cell stress markers neither in the whole cohort nor in patients with grade 3-4 GvHD. Patients with or without statins had similar NRM, relapse rates and overall survival. **Conclusions.** This study supports the hypothesis that steroid-refractory GvHD is associated with progressive microangiopathy. Statins, although reported to have protective effects on endothelial cells, were inefficient to alleviate endothelial stress in this context and accordingly, did not change the outcome of acute GvHD patients.

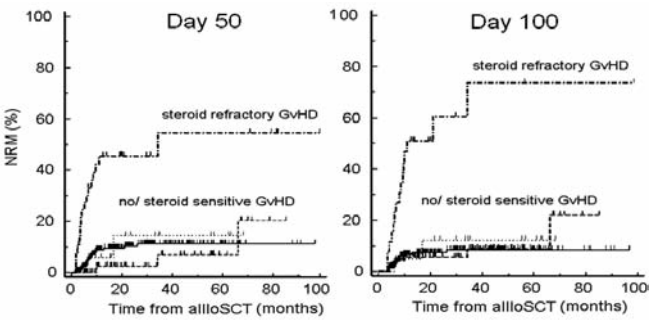

Figure 1. NRM rate by GvHD.

0448

COMPARISON OF IMMUNE RECONSTITUTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH FLU-TBI VERSUS TLI-ATG CONDITIONING

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Background. The impact of the type of reduced intensity conditioning regimen used on immune recovery after allogeneic hematopoietic cell transplantation (allo-HCT) is poorly determined. **Aims.** We analyzed immune reconstitution in patients enrolled in a BHS-HCT sponsorised randomized study comparing two non-myeloablative conditioning regimens for allo-HCT for which cell samples were prospectively collected. **Patients and Methods.** The conditioning regimen consisted of either 2 Gy TBI with 90 mg/m² fludarabine (=TBI arm, n=21), or 8 Gy TLI plus thymoglobulin (ATG) 7.5 mg/kg (=TLI arm, n=19). Median ages at HCT were 59 yrs and 61 yrs in the TBI and TLI arms, respectively. Immune reconstitution was assessed by flow-cytometry phenotyping, signal joint T-cell Receptor Excision Circle (sjTREC) quantification, and T-cell spectratyping. Written informed consent has been obtained for each patient included. **Results.** Absolute T cell counts were lower in the TLI arm than in the TBI arm on day 28 after HSCT (P=0.04) but not thereafter. Further, B cells, as well as CD4+, CD4+CD45RA+ and CD4+CD45RO+ T cell reconstitution lagged behind in the TLI arm compared to the TBI arm the first year after HCT (B cells: p=0.0295 and others: p>0.0001). In contrast, reconstitution of CD8+ T cells, NK cells, Tregs and iNKT cells were similar in the 2 groups. For the thymic function, while sjTREC levels were higher in the TBI arm than in the TLI arm on day 100 (P=0.002) and on day 365 (not significant) after HCT, the increase in sjTREC levels from day 100 to day 365 was similar in the 2 groups of patients. The diversity of the TCR repertoire was similar in the 2 groups of patients on day 100 after HCT. Finally, we found that ATG persists in patients up to 17 days after allo-HCT in TLI patients (median of [ATG] at day 17=0.62 mg/l and for one patient at day 20=0.53). **Conclusions.** These preliminary results suggest that ATG may be responsible for the delay of immune reconstitution of CD4+ T cells in the TLI arm. Furthermore, ATG probably destroyed grafted sjTREC+ T cells, explaining the difference of sjTRECs level at days 100 and 365 between the two groups while sjTREC increment from day 100 to day 365 was similar in the 2 groups. Finally, TLI conditioning has no impact on immune regulatory populations (Treg and iNKT) after the transplantation.

0449

UP-FRONT AUTOLOGOUS STEM CELL TRANSPLANTATION BRINGS SURVIVAL BENEFIT TO PATIENTS WITH POOR-RISK FOLLICULAR LYMPHOMA

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Background. Intensive frontline therapy of follicular lymphoma remains controversial. But in the era of conventional chemoimmunotherapy, long-term progression-free survival of high-risk patients is poor and drops to only 30% in 5 years. **Aims.** A retrospective analysis of FL patients with unfavorable prognostic features receiving up-front autologous stem cell transplantation and comparison with anthracycline-based regimens. **Methods.** We studied 164 patients with newly diagnosed FL who met the GELF criteria. Forty (24%) were treated with ASCT (conditioning BEAM 200) due to high-risk disease (FLIPI, B2M, s-TK, bulky). Frontline therapy with chemotherapy only (CHT) was administered to 124 (76%) patients: CHOP/CHOP-like regimen to those with low-risk FL (n=95) and intensive protocol (Promace-Cyta-BOM/adequate intensity protocol) to intermediate-risk patients. Rituximab was added to frontline therapy in 60% and 56% of cases in ASCT and CHT group, respectively (p=ns). Maintenance immunotherapy was applied in 30% (ASCT) and 41% (CHT), p=0.22. ASCT patients shared more unfavorable prognostic features than CHT group: H-FLIPI (66% vs 34%, p=0.001), bulky disease >7cm (81% vs 51%, p=0.001), advanced clinical stage (95% vs 80%, p=0.02), elevated B2M (64% vs 39%, p=0.007). ASCT patients were significantly younger (median age 46 vs 57 years, p<0.01). **Results.** Complete remission rates were 93% and 81% in ASCT and CHT group, respectively (p=ns). Molecular CR was achieved in 89% of ASCT patients and 81% of CHT group (p=ns). After a median follow-up of 87 months (7.3 yrs), 29/40 (73%) ASCT patients are alive in the 1st CR as compared with only 68/124 (55%) in CHT group. Five-year PFS was 76% (95% CI 0.62-0.90) and 56% (95% CI 0.47-0.66, p=0.018) in ASCT and CHT group, respectively. Five-year overall survival of 95% (95% CI 0.88-1.00) in ASCT group was superior to that of 84% (95% CI 0.77-0.91, p=0.024) in CHT arm. Subanalysis of 93 patients treated with rituximab showed higher proportions of relapses and deaths in CHT group (22 events in 69 patients) than in ASCT group (4 events in 24 patients, p=0.048). Five-year PFS was higher in ASCT (86%, 95% CI 0.71-1.00) than CHT group (63%, 95% CI 0.51-0.76). Despite 23% difference in 5-year PFS in favor of ASCT, statistical significance was not achieved (p=0.08). There was no difference in OS in rituximab treated patients; only 2 (8%) ASCT patients died as compared to 10 (14%) deaths in CHT group (p=0.13).Long-term toxicity in ASCT arm is acceptable. So far, no secondary MDS or AML have been observed; during the follow-up (median 100 months), only 4 patients died (2 lymphoma, 1 prostate cancer, 1 pneumonia). **Conclusions.** Frontline ASCT is very effective in high-risk FL patients. This approach overcomes multiple negative prognostic features of the disease and leads to superior survival compared to lower-risk patients treated with chemotherapy only.Supported by grants from Czech Ministry of Education (MSM 6198959205) and Faculty of Medicine and Dentistry, Palacky University Olomouc (LF-2011-006).

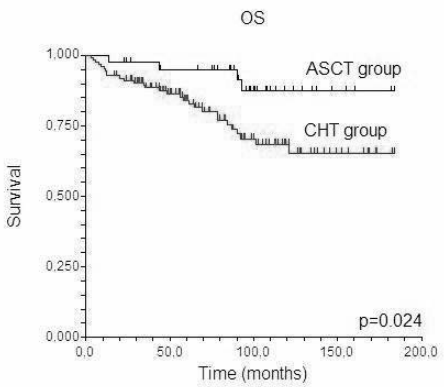

Figure 1.

0450

SINGLE-INSTITUTION LONG-TERM OUTCOMES FOR PATIENTS RECEIVING NONMYELOABLATIVE CONDITIONING HEMATOPOEITIC CELL TRANSPLANTATION FOR CHRONIC LYMPHOCYTIC LEUKEMIA AND FOLLICULAR LYMPHOMA

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Background. Chronic lymphocytic leukemia (CLL) and follicular lymphoma (FL) are indolent lymphoproliferative malignancies that can transform into more aggressive high-grade non-Hodgkin lymphomas. In this group of often elderly and heavily pretreated patients, the introdution of nonmyeloablative conditioning (NMC) has made allogeneic hematopoietic cell transplantation (HCT) an efficacious and safe treatment option. The results for NMC-HCT in FL have been encouraging with long term overall survival up to 85% and low relapse rates. For patients with transformed FL, a worse prognosis than in non-transformed patients has been reported. Patients with CLL generally have a lower overall survival and a higher relapse rate after NMC-HCT. **Aims and Methods.** The aim of this study was a retrospective analysis of treatment outcome after NMC-HCT in patients with FL and with CLL. Data from all patients treated in our instutition with NMC-HCT for CLL and FL from 2000 to 2011 were identified. Patient pre-HCT characteristics and outcome were analysed. **Results.** In a cohort of 85 patients (45 with CLL and 40 with FL) we observed five years overall survival (OS) and progression free survival (PFS) of 53% and 38% in the CLL group and 81% and 76% in the FL group. In the CLL group the estimated four years OS and PFS were both 86% for patients in CR at HCT and five years OS and PFS were 50% and 33% for patients not in CR at HCT. In the FL group the five years OS and PFS were 96% and 91% for patients in CR at HCT and 67% and 62% for patients not in CR at HCT. Within the FL group sixteen patients had at one or more time points in their disease history had transformed FL. The five years OS was almost identical in patients with transformed and non-transformed FL, 83% and 78% respectively (figure). **Conclusions.** Our study supports that NMC-HCT is a safe and efficacious treatment in elderly, heavily pretreated patients with FL and CLL. Especially patients with FL, and also transformed FL, seem to have a great benefit of NMC-HCT. CR at the time of HCT is an important prognostic factor.

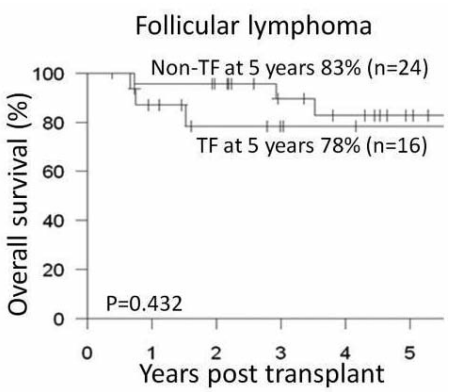

Figure 1.

0451

THROMBOTIC AND HEMORRHAGIC EVENTS AFTER ALLOGENEIC HEMATOLOGIC STEM CELL TRANSPLANTATION: ANALYSIS OF 443 CONSECUTIVE PATIENTS IN A SINGLE INSTITUTION

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Background and Aims. In recent years, it has been recognized that the incidence of thromboembolic events (TEEs) in patients undergoing allogeneic stem cell transplantation (HSCT) is similar to that of patients with solid tumours. In this setting, a thromboprophylaxis strategy could be effective in preventing

TEEs in selected allogeneic HSCT recipients. However, TEEs events have been separately analyzed without taking into consideration the increasing risk of bleeding in allogeneic HSCT recipients. Here, we report the largest study for competing risk of thrombosis and bleeding in allogeneic HSCT performed to date. **Methods.** We conducted a retrospective analysis of 443 consecutive patients (age > 18 years-old) who had undergone allogeneic HSCT between 1995 and 2011 at the Hospital Universitario de Salamanca. Twelve patients who received prophylaxis or therapeutic anticoagulation at the time of HSCT admission were excluded. The primary end-points of the study were the incidence, risk factors and clinical impact of post allogeneic HSCT TEEs and hemorrhages. **Results.** Median follow-up was 20 months. The incidence of venous TEEs, arterial TEEs and bleeding episodes were 6.03%, 1.9% and 32.5 % respectively. The probability of post allogeneic HSCT venous TEEs, arterial TEEs and bleeding at 14 years were 20.7%, 6.6% and 36.6 %. The median time from allogeneic HSCT day 0 to diagnosis of venous TEEs and first bleeding episode were 211 days (range, 9 to 4080 days) and 60 days (range, 2 - 3660 days). The development of extensive chronic graft versus host disease (GVHD) was the only risk factor for the occurrence of venous TEEs post allogeneic HSCT [OR = 2.85, 95% CI (1.20 - 6.80)]. Only a trend to higher rate of arterial events was observed for patient with a BMI > 25 [OR = 2.85, 95% CI (0.81 - 12.34, p = 0.098)]. Advanced disease, ablative conditioning regimen, umbilical cord stem cell transplantation, anticoagulation after HSCT, grade acute III - IV GVHD and thrombotic microangiopathy (TMA) retained their association with bleeding events in the multivariate analyses. The median OS of patients with bleeding episodes was only 15 months, as compared to patients without bleeding episodes (122 months) (p<0.001) (Figure 1A). In the multivariate analysis, bleeding events after allogeneic HSCT retained their independent prognostic value (HR: 3.18 95% CI: 2.2). However, there was no significant difference in OS between allogeneic HSCT recipients with TEEs and allogeneic HSCT recipients without TEEs (p = 0.856) (Figure 1B). **Conclusions.** This study evaluates the incidence, risk factor and mortality of thrombosis and hemorrhage of patients undergoing allogeneic HSCT with the longest follow-up to date. The incidence of VTE was relatively high, during both early and late phase of HSCT but did not influence survival. The most important risk factor for developing this complication was the presence of extensive chronic HSCT. In contrast, bleeding which was more frequent is associated with increased mortality. The principal risk factors for bleeding were myeloablative conditioning, umbilical cord transplantation, severe thrombocytopenia after day +28 and development of grade III-IV acute GVHD or TMA.

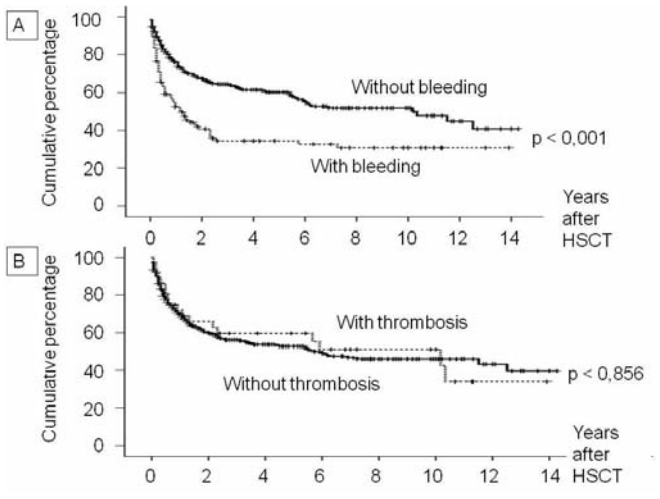

Figure 1. Overall survival of patients with and without bleeding (A), and patients with and without thrombosis (B) after allogeneic HSCT.

0452

USE OF A BIOSIMILAR GRANULOCYTE COLONY STIMULATING FACTOR (GCSF) FOR PERIPHERAL BLOOD STEM CELL MOBILISATION PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANTATION: AN ANALYSIS OF MOBILISATION AND ENGRAFTMENT

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Background. Mobilisation of peripheral blood haematopoietic progenitor cells (PBPCs) has become a standard procedure for patients prior to an autologous stem cell transplant (ASCT). In September 2008 a biosimilar GCSF (Ratiograstim) was granted an EU licence for use in mobilisation of PBPCs. Ratiograstim was incorporated into clinical use for PBPC mobilisation in the Wessex Blood and Marrow Transplantation Programme from November 2008, replacing the previous form of GCSF. **Aims.** An audit of stem cell harvests was performed for the periods Jan 2007- Sept 2008 (standard GCSF) and Jan 2009-Dec 2011 with the biosimilar GCSF (Ratiograstim), comparing the CD34+ maximum predictor, total yield and number of days required for collection. In addition, we have compared engraftment data for the two groups following high dose chemotherapy and ASCT using the harvested stem cells. **Methods.** Data were retrospectively collected for 154 patients undergoing PBPC harvest at University Hospital Southampton (UHS) from Jan 2009-Dec 2011 using the biosimilar GCSF; 92 patients who underwent the procedure Jan 2007-Sept 2008 were used as a comparator group mobilised with a standard GCSF. The protocols for stem cell priming were essentially the same throughout. The p values were calculated using two tailed t tests. **Results.** Of the patients in the period Jan 2007-Sept 2008 (standard GCSF) 42 patients had myeloma, 49 lymphoproliferative diseases (LPDs) and one non-haematological malignancy. Of the patients in the period Jan 2009-Dec 2011 (biosimilar GCSF) 76 patients had myeloma, 71 LPDs, six non-haematological malignancies and one non-malignant condition. With one exception, all the patients with myeloma were primed with cyclophosphamide and GCSF. The LPD and non-haematological patients received a variety of regimens. The mean age of patients was 53.1 (±26.5, two standard deviations) years for the Ratiograstim group and 54.1 (±.23) years for the standard GCSF group.The mean number of sessions required for harvest was 1.4 (±1.2) for the Ratiograstim group and 1.4 (±1) for the standard GCSF group, with 66% of both groups completing their harvest in one session. The mean CD34x10^6/kg count per complete collection was 6.6x10^6 (±13.8) for the Ratiograstim group and 6.5x10^6 (±14.6) for the comparator group (p=0.9).Of those patients audited, 111 patients in the biosimilar GCSF group, and 57 in the standard GCSF group proceeded to high dose therapy and ASCT. Engraftment data were compared for the two groups until day of discharge. The mean number of days to a neutrophil count of 0.5x10^9/L was 13 (±6) in the Ratiograstim group and 13 (±4.8) in the standard group (n=107 and n=57, p=0.6). The mean number of days to a platelet count of >20x10^9/L was 13 (±8) in the Ratiograstim group (n=90) and 14 (±8) in the standard group (n=55)(p=0.4). **Conclusions.** This is the largest comparison of a biosimilar GCSF and standard GCSF used to mobilise PBSCs and shows no statistically significant difference between standard and biosimilar GCSF for the key parameters measured for PBPC harvest. In addition this study has not found a significant difference in time to engraftment between the two groups.

0453

CD3/T REGS RATIO IN DONOR GRAFT DOES NOT IMPACT ON RELAPSE FREE SURVIVAL WHILE PROTECTING AGAINST AGVHD

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Introduction. The therapeutic efficacy of allogeneic stem cell transplantation (alloSCT) for hematological malignancies relies largely on the graft versus leukemia (GVL) effect exerted by the donor CD3 cells, but an uncontrolled graft-versus-host-disease (GVHD) bears a risk of complications. On the other hand, T regs cells (CD4+CD25^{high} Foxp3+) are believed to maintain tolerance and to inhibit GVHD after alloSCT; also, the Foxp3 gene encodes a transcription factor that is a key for thymic development, so T regs cells could also preserve an optimal microenviroment for the reconstitution of functional immunity after alloSCT. **Patients and methods.** In this study we analyzed the graft CD3+/Tregs ratio (gCD3/Tregs R) and determined its impact on acute GVHD (aGVHD), immunological recovery and survival rates (OS and RFS) after myeloablative alloPBSCT. We analyzed 75 consecutive patients transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor (n=50) or an HLA identical unrelated donor (n=25); diagnoses were acute

myeloid leukaemia (n=62), acute lymphoblastic leukaemia (n=13).The median CD3+ and Tregs dose administered was 238 (range (r): 67-550) and 12,5x10^6/Kg (r: 2-21), respectively; the median gCD3/Tregs R was 19 (r: 8-250). Patients were subdivided into a high gCD3/Tregs R (>=36) group (n= 31) and a low gCD3/Tregs R (<36) group (n=44). **Results.** The incidence of aGVHD (grade II-IV) in the low gCD3/Tregs R (LR) group was lower than in the high gCD3/Tregs R (HR) group (9/44 or 20% vs 24/31 or 77%, p<.001). At multivariate logistic regression, gCD3/Tregs R was correlated both with aGVHD (Odds Ratio (OR): 2.60, 95% CI (1.35,4.90), p=.05) and with CMV infection/disease (OR: 2.45, 95% CI (0.8,5.50), p=.05). The mean OS time was significantly affected by gCD3/Tregs R (32 vs 67 months in the H and LR group, respectively, p<.001); on the contrary, there was no difference with regard to RFS (mean RFS time: 64 vs 65 months; p=.90). **Conclusions.** Taken together, our data may suggest that Tregs content is able to mediate protective effects against aGVHD, while maintaning GVL effect, as suggested by RFS median time equality between H and LR group.Larger studies are needed in order to understand the real contribution of gCD3/Tregs R on control of both GVHD and leukemia. Trascrizione foneticDizionario - Visualizza dizionario dettagliato

0454

HIGH 7 YEAR-SURVIVAL RATES IN ADULT LYMPHOBLASTIC LYMPHOMA AFTER TREATMENT WITH A NORWEGIAN INTENSIFIED ALL INDUCTION, HIGH-DOSE BEAM, AUTOLOGOUS STEM CELL SUPPORT AND MEDIASTINAL RADIATION THERAPY

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Lymphoblastic lymphoma is a highly aggressive disease with reported overall (OS) and event free (EFS) survival of 46-72%. Many achieve CR by acute lymphoblastic leukaemia (ALL) -like regimens, but often progress or rapidly relapse.Since 1999 we have treated adult patients with T- (T-LL) or B- (B-LL) lymphoblastic lymphoma with 4 months of the Norwegian intensified ALL regimen adapted from the MRC Leukaemia Unit , London, as induction and first consolidation, and if in CR/CRu, followed by high-dose BEAM with autologous stem cell support (ASCT). Patients with mediastinal tumour at diagnosis, received radiotherapy 23.4 - 30 Gy at end of treatment.During 1999-2010 twenty five patients were admitted with lymphoblastic lymphoma to The Norwegian Radium Hospital: median age 32.5 years (15-65), male/female 60/40%, T-LL/B-LL 76/24%, stage I-II/III-IV 24/76%, B-symptoms 32%, high LDH 68%, bulky tumour (≥10 cm) 48%, minimal (≤25%) bone marrow involvement 20%, significant bone marrow involvement (>25%) 4%, CNS involvement 12%.Induction therapy included prednisone, vincristine and L-asparaginase, with doxorubicine weekly for three weeks and cyclophosphamide weekly for three weeks. Consolidation was daunorubicin, cytarabine and 6-thioguanine (RAT), followed by 2 doses of high-dose methotrexate (MTX) and continuous 6-mercaptopurine orally for 7 weeks. Intrathecal MTX was administered x 7. Stem cells were harvested after RAT.Four patients received other drugs for one course prior to inclusion. One patient was treated with enforced consolidation with MTX and Ara-C due to cranial nerve involvement and young age (15yrs). Another young (15yrs) patient was treated according to the BFM-95 regimen prior to BEAM and ASCT due to solely involvement of the central nervous system.23 of 25 patients completed ASCT. 16 of 17 patients with mediastinal involvement received radiotherapy. One patient with t(9;22) (BCR-ABL translocation) and bone marrow involvement, who was considered too fragile for allogeneic transplantation, received imatinib as maintenance treatment post-ASCT.At median follow up of 7 years (1-153 months) of the whole cohort, OS and EFS survival are 84% and 76%, respectively. 19 of 25 are alive in first CR. Two patients died due to toxicity (veno-occlusive disease and cerebral sinus venous thrombosis), one due to progressive disease (T-LL) prior to ASCT and one from relapse (T-LL) two years after treatment. Two patients (B-LL), who relapsed at 5 and 25 months of follow up, respectively, are in second CR after reinduction with hyperCVAD and myeloablative allogeneic stem cell transplantation. In 2 of 4 relapsing patients the disease returned as lymphoblastic leukaemia.No secondary cancers are reported. None has developed heart failure or permanent clinically reduced lung function. Disabling polyneuropathy is seen in two patients, both highly overweight at diagnosis. Surgical treatment due to osteonecrosis in large joints is done in three patients, all males. Three females got pregnant after treatment, one by egg donation and four males have become fathers, either by cryopreserved sperm or fresh samples. **Conclusions.** We report 84% OS and 76% EFS in lymphoblastic lymphoma 7 years after the Norwegian intensified ALL induction, high-dose BEAM with ASCT and radiotherapy consolidation to the mediastinum in a population-based cohort.

0455

HIGHER INCIDENCE OF SEVERE PNEUMONIA AFTER CONDITIONING WITH BUSULFAN-FLUDARABINE COMPARED TO BUSULFAN-CYCLOPHOSPHAMIDE IN A PERSPECTIVE, RANDOMIZED STUDY

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Background. Cytarabine-busulfan-phosphomide (CBuCy) regimen has been the most frequent types of myeloablative conditioning used in allogeneic hematopoietic stem cell transplantation (HSCT) in our center. However, its non-hematologic toxicities and related mortality were still concerned. Fludarabine showed its safety and efficacy in non- myeloablative conditioning regimens. However, whether cyclophosphomide in CBuCy could be substituted with fludarabine is unknown. Therefore, we designed the perspective, randomized, comparative study. **Aims.** To investigate whether CBuCy could be substituted with Cytarabine - busulfan - fludarubine (CBuF) in HSCT from identical siblings. **Methods.** The elements of conditioning CBuF and CBuCy were described in Table 1. 105 patients were enrolled,52 in CBuF group and53 in CBuCy group. At baseline both groups were matched for characteristics demographics and disease. Surviving patients were followed up to 20 February, 2012. The primary study endpoints were overall survival (OS), engraftment, and acute GVHD. The secondary endpoints were leukemia-free survival (LFS), relapse, non-relapse mortality (NRM), conditioning toxicity and chronic GVHD. **Results.** All patients in CBuF achieved neutrophils and platelets engraftment. Fifty-two (98.1%) patient from the CBuCy group achieved neutrophils engraftment and 51 platelets engraftment (96.2%). There were 43 and 46 patients from the two groups did not develop acute GVHD. The incidences of acute GVHD grade 1-4 (P = 0.577) or 3-4 (P = 0.189) were not significantly different. Ninety-eight patients (49 in each group) were evaluated for occurrence of chronic GVHD. No difference in the incidence of chronic GVHD (P = 0.590), extensive GVHD (P = 0.241) was found. Up to 20th of February, 2012, 37 patients from CBuF group and 36 from CBuCy survived. Thirty-two patients died, 19 died of non-relapse (NRM) causes and 13 died of recurrence of disease. The 2 yr probability of OS was 70.5% and 69.1% (P = 0.912), and LFS was 61.4% and 64.5% (P = 0.714), respectively. No difference of NRM (P = 0.192) or relapse (P = 0.415) between the two groups was found.Within the first 2 weeks after transplantation, there was no difference in occurrence of hematological toxicities, diarrhea, liver or renal dysfunction. In CBuF group, lower incidence of nausea and vomiting (9.6% vs 28.3%, P = 0.024) and higher incidence of mucosal damage (65.4% vs 32.7%, P = 0.002) was found compared to CBuCy group.Thirty patients developed pneumonia, 18 of these were severe and accompanied with respiratory failure. Ten patients died of severe pneumonia. The cumulative incidence of severe pneumonia in CBuF group was higher than that in CBuCy group (30.6% vs 13.1%, P = 0.05), however, no difference in pneumonia related mortality was found (P = 0.057). The study started from March of 2009, and was closed on 20th, August of 2010 due to this severe side effect. **Conclusions.** This is the first randomized, perspective study to compare CBuF and CBuCy for allogeneic HSCT. It should be cautious to substitute cyclophosphomide with Fludarabine in the context of cytarabine/busulfan/phosphomide based conditioning due to the pulmonary morbidity.

Table 1. Conditioning regimens for allo-HSCT from identical siblings.

HSCT(d)	Modified BUCY	Bu-Fludarabincin
-10	Hu 80mg/kg	Hu 80mg/kg
-9	Ara-C 2 g/m^2	Ara-C 2 g/m^2
-8	Bu 3.2 mg/kg, iv	Bu 3.2 mg/kg, iv
-7	Bu 3.2 mg/kg, iv	Bu 3.2 mg/kg, iv
-6	Bu 3.2 mg/kg, iv	Bu 3.2 mg/kg, iv
		Flu 30 mg/m^2, iv
-5	Cy 1.8 g/ m^2	Flu 30mg/m^2, iv
-4	Cy 1.8 g/ m^2	Flu 30mg/m^2, iv
-3	Me-CCNU 250 mg/m^2	Me-CCNU 250 mg/m^2
		Flu 30mg/m^2
-2		Flu 30mg/m^2

0456

THE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN KOREAN CHILDREN WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background. Although chemoimmunotherapy-based treatments have improved the survival of patients with hemophagocytic lymphohistiocytosis (HLH), outcomes of the patients with familial hemophagocytic lymphohistiocytosis (FHL), or refractory or reactivated HLH are still unsatisfactory. **Aims.** This study was conducted to evaluate the prognostic factor and treatment outcome of Korean children with HLH who underwent allogeneic hematopoietic stem cell transplantation (HSCT). **Methods.** We analyzed the data of Korean nationwide HLH registry. Retrospective nationwide data recruitment for the pediatric HLH patients diagnosed between 1996 and 2011 was carried out by the Korea Histiocytosis Working Party. **Results** Thirty patients who received allogeneic HSCT among the total of 279 enrolled children with HLH were analyzed. Conditioning regimen included busulfan/cyclophosphamide/etoposide with or without antithymocyte globulin (ATG) (n = 20), busulfan/fludarabine/ATG (n = 3), fludaraibe/melphalan with ATG or alemtuzumab (n = 4), and others (n = 3). The probability of 5-year overall survival (OS) after HSCT was 75.4% with a median follow-up of 72 months. The reasons for HSCT were active disease after 8 weeks of initial treatment (n = 10), reactivated disease (n = 10), and FHL (n = 10). Eight of the 10 patients with FHL were shown to have mutations of the *UNC13D*. Twenty-three patients are currently alive without disease after HSCT, whereas 6 patients died of treatment-related events at early post-transplant period, and 1 patient died of reactivation at 1 year post transplantation. The survival of patients who were transplanted because of active disease after 8 weeks of initial treatment was inferior compared to those patients who had inactive state at that time (62.2% vs. 100%, respectively, P = 0.031). Patients who received cord blood graft (n = 6) showed significantly inferior OS than those who received bone marrow (n = 15) or peripheral blood graft (n = 9) (33.3% vs. 80.0% vs. 100%, P = 0.025). The 5-year OS rate after HSCT according to the donor type was 75.0% for the matched related donors (n = 8) and 74.8% for the unrelated donors (n = 22) (P = 0.813). Other variables such as age, CNS involvement at the time of diagnosis, the etiology of HLH (familial or secondary), and the conditioning regimens had no influence on the survival outcome of the HLH patients who underwent HSCT.5. **Conclusions.** HSCT improved the survival of the patients who had familial, reactivated, or severe and persistent secondary HLH in the Korean nationwide HLH registry. The disease state after initial treatment and the stem cell source were the important prognostic factors. The impact of the intensity of the condition regimen and the graft source on the outcome is required to be evaluated in the prospective trial.

Bleeding disorders

0457

NINE NOVEL ADAMTS13 MUTATIONS IN CONGENITAL PREGNANCY ASSOCIATED ADULT ONSET TTP

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ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domains 13) deficiency is associated with thrombotic thrombocytopoenia pupura (TTP), a rare, life threatening thrombotic microangiopathic anaemia. TTP may be acquired, usually due to the presence of autoantibodies, or congenital. The congenital form can present in childhood, adolescence or adulthood, but in adults usually occurs secondary to haemostatic challenge. The differences in age of onset are likely to result from the individual repertoire of mutations in the ADAMTS13 gene, located on Chromosome 9.DNA sequencing was performed on nine patients with pregnancy associated, adult onset TTP, two of whom were sisters. Eight of the patients were Caucasian and one of Asian origin. All the patients presented with a markedly reduced ADAMTS13 activity of < 5% (FRETs NR 60-123%) and negative for anti-ADAMTS13 IgG autoantibody. Using population data from the 1000 Genomes Project and Hapmap we confirmed the presence of 3 single nucleotide polymorphisms (SNPs), 10 synonymous SNPs, 5 missense mutations, 4 frame shift mutations and 4 intronic mutations.Six novel exonic mutations and 3 novel intronic mutations were identified. These included two exonic missense mutations: R497C and R1219Q. Four frame shift mutations were found: p.A111QfsX18, p.N667TfsX31, p.M486VfsX47 and p.I1339SfsX21, one of which occurred in both of the two siblings. The intronic mutations detected were c.L987+11, c.L1786+90 and c.Q1245+81. Some SNPs had a higher prevalence in our patients than anticipated from normal population frequencies, for example R7W is present in 7% and A1033T is present in 3% of the general Caucasian population, but were found in 7 of the 8 Caucasian patients studied. Exons 4,6,13,15 and 24, exclusively in our cohort, demonstrated several differences from wild type, whereas exons 2,3,7,9,10,14,20,22,25,27 showed no variation from wild type in any of the patients investigated. Notably, seven of the nine patients had both the R7W and A1033T SNPs in conjunction with the R1060W mutation. The patients' full mutational profile (including synonymous mutations) was examined in the context of their clinical features. One patient with a moderate clinical profile; only one heterozygous missense mutation (R1060W) and relatively few exonic differences from wild type (R7W, A900V, A1033T, V970V) however had three intronic mutations. It is not yet understood how these may interact to influence the clinical picture, or how their position on the allele may contribute. In conclusions in conjunction with further novel mutations in our cohort, we have also documented a higher frequency of SNPs and intronic mutations in association with synonymous SNPS, further work will focus on how these new findings result in changes in expression and function of ADAMTS13 in relation to TTP.

0458

PLASMA CONCENTRATION OF PROTEIN Z AND PROTEIN Z-DEPENDENT PROTEASE INHIBITOR IN PATIENTS WITH HAEMOPHILIA A

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Background. The potential role of alterations in protein Z concentrations in the pathogenesis of coagulation has been investigated in several studies which, however, yielded conflicting results. Protein Z deficiency may induce bleeding as well as prothrombotic tendencies and it might occur as an inherited disorder. **Aims.** The purpose of the present study was to explore the concentration of protein Z and protein Z-dependent protease inhibitor in patients with haemophilia A. Additionally; it examined the correlation between PZ/ZPI plasma concentration and bleeding rate and joint bleeding per year. **Materials and Methods.** The study was based on fifty male patients: 25 with severe, 15 with moderate and 10 with a mild form of haemophilia A. Median age at the time of samples taking was 29 (range 19-55 years). None of these patients had undergone prophylaxis of bleeding with FVIII (factor VIII) before the study. All studied subjects received FVIII on demand, doses depending on the kind of bleeding. Haemophilia A patients with confirmed hepatitis B or C and with inhibitor of factor VIII had been excluded from the study. The control group consisted of 90 healthy male blood donors at median age of 30 (range 19-52). The yearly bleeding rate and joint bleeding rate were calculated on the basis of data from medical records and home-treatment reports from 2005 to 2011.Quantitative assessment of protein Z and ZPI in the plasma was performed using commercial test (Asserachrom Protein Z Elisa Kits, Diagnostica Stago, France) with expected mean value (±SD), 1.56±0.61µg/mL) and Enzyme-linked Immunosorbent Assay Kit for Protein Z Dependent Protease Inhibitor (ZPI) (USCN Life Science Inc, China). Quantitative determination of FIX in the plasma was done using Stago® Deficient IX (Stago,France), with expected normal range of factor IX between 60-150%. Active form of FVIII was tested by means of standard methods. **Results.** In haemophilia A patients mean plasma concentrations of PZ and ZPI were significantly higher than in healthy individuals: PZ (1.87±0.68 µg/mL vs 1.49±0.54 µg/mL) and ZPI (5.02±1.11 µg/mL vs 4.22±0.55 µg/mL), with p=0.02 and p=0.03, respectively. In the subgroup with severe haemophilia A, an in-depth analysis revealed a tendency to modulating effect of the PZ (r=-0.53; p=0.072) and a statistically significant one in the case of ZPI (rho=-0.79, p=0.002) on the bleeding rate. It simultaneously disclosed a statistically significant correlation between the number of bleeds to the joints (20.18±14.1), PZ (r=-0.72; p=0.04) and ZPI (rho=-0.88, p=0.001). With reference to this particular group of patients, the study also showed some other statistically meaningful correspondences: between PZ and ZPI (rho=0.65, p=0.02), PZ and FIX (r=-0.61, p=0.04), as well as ZPI and FVIII (rho=0.78, p=0.002).No such link in patients with mild and moderate haemophilia A was established. **Conclusions.** Despite the fact that FVIII deficiency is undoubtedly the main mechanism of bleeding in haemophilia A patients, the activity of PZ/PZI complex may play some modulating role in the matter. Further research is required to fully elucidate the haemostatic role of ZP/ZPI complexes in haemophilia A patients.

0459

WITHDRAWN

0460

LOCAL EXPERIENCE IN REVERSAL DABIGATRAN USING ACTIVATED PROTHROMBIN COMPLEX CONCENTRATES (FEIBA)

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Background. Dabigatran etexilate (Pradaxa, Boehringer Inhgelheim) is a prodrug of dabigatran, a reversible direct inhibitor of thrombin that exhibits its effect by binding directly to the active site of thrombin. The major advantage of dabigatran over warfarin is that it produces a predictable pharmaco-dynamic effect which renders routine laboratory monitoring unnecessary. Several studies demonstrated that the efficacy of dabigatran is at least comparable to that of current therapies in preventing stroke related to atrial fibrillation and venous thrombosis following hip and knee replacement surgeries.Like other anticoagulants, Dabigatran is associated with a risk of major bleeding with an expected rate of 1-2% per annum receiving therapeutic anticoagulation. The optimal management of a major bleeding complication occurring in patients receiving Dabigatran remains uncertain, and current recommendations regarding the use of reversal agents are based on a combination of pre-clinical data, anecdotal case reports and the absence of effective alternatives. **Aims.** To determine the usefulness of the CAT in determining the effect of dabigatran at therapeutic concentrations, and to measure the effect of activated prothrombin complex concentrate (FEIBA®, Baxter AG, Vienna, Austria) in reversing the haemostatic defect induced by dabigatran. **Methods.** The thrombin generation assay was performed in platelet poor plasma (PPP) using the Calibrated Automated Thrombogram (CAT) (Thrombinoscope). Commercial plasmas containing a range of concentrations of dabigatran (30 to 500 µg/L) were tested. Different therapeutic concentrations of FEIBA (0.2 - 3 U/ml) were added to a test plasma containing approximately 200 µg/L dabigatran. Commercial PPPLOW reagent (1 pM Tissue factor with 4 µM phospholipids) and Fluorogenic substrate (FluCa) were used (Thrombinoscope) according to manufacturer's manual. **Results.** Dabigatran induced a concentration-dependent increase in lag time and time-to-peak, as well as a decrease in peak height and ETP at higher concentrations (>130 µg/L). Addition of FEIBA to the test sample decreased the lag-time and time-to-peak in a concentration-dependent manner between 0.2 and 1U/ml FEIBA, with no further decrease with 2 and 3 U/ml FEIBA. At all concentrations both times remained prolonged in comparison to normal. Peak height and ETP was increased to normal values by 0.2 U/ml FEIBA, with further increases above normal up to 1 U/ml, and no further increase with higher concentrations of FEIBA. **Conclusions.** Our results demonstrated that FEIBA partially reversed dabigatran mediated inhibition of thrombin generation (i.e. shortening of delay in lag time and increasing peak response). Future experiments should be conducted to determine the in-vivo effect of FEIBA in reversing dabigatran in overdosed patients, correlated with clinical outcomes.

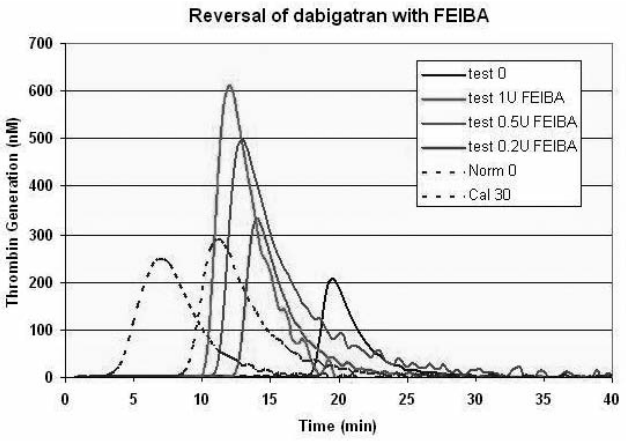

Figure 1. Partial reversal of delay in lag time in Thrombinoscope by Feiba in test sample.

0461

A NOVEL FLOW CYTOMETRY-BASED PLATELET AGGREGATION ASSAY THAT DISCRIMINATES BETWEEN GLANZMANN THROMBASTENIA AND LAD-III SYNDROME.

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Background. The main function of platelets is to maintain normal hemostasis. Several platelet function tests have been developed to date to be used in the clinic and in experimental animal models. In particular, platelet aggregation is routinely measured in an aggregometer, which requires normal platelet counts and significant blood sample volumes, making difficult analyses in thrombocytopenic patients or infants. For the same reason, analysis of platelet aggregation in small rodents can be tedious. Patients with Glanzmann thrombasthenia or Leukocyte Adhesion Deficiency-III syndrome (LAD-III or LAD-1/variant) present with increased bleeding tendency because of the lack or dysfunction of the fibrinogen receptor GPIIb/IIIa (integrin αIIbβ3), respectively. Although the bleeding disorder is more severe in LAD-III patients, classic aggregometry or perfusion of Glanzmann or LAD-III platelets over collagen-coated slides under physiologic shear rate does not discriminate between these 2 conditions because it requires functional integrin αIIbβ3 as a readout. **Aims.** To develop a novel test of platelet aggregation, in which lower platelet counts or volumes can be used, and that would allow dissection of single receptor contribution to the platelet aggregation process. **Methods.** Platelet labeling with fluorescent dyes or conjugated antibodies, flow cytometry. **Results.** We have developed a novel flow cytometry test of platelet aggregation, in which 10-25-fold lower platelet counts or volumes can be used, either from platelet-rich plasma or whole blood (including murine embryonic blood) from human subjects or mice. Furthermore, this novel test is able to discriminate between Glanzmann thrombasthenia and LAD-III aggregopaties. We were able to measure collagen-dependent aggregate formation in Glanzmann platelets, whereas LAD-III platelets were not able to form aggregates upon this stimuli. These aggregates required functional GPIa/IIa (integrin α2β1) instead of integrin αIIbβ3, thus explaining the clinically more severe bleeding manifestations in LAD-III patients, in which all platelet integrins are functionally defective. These findings provide genetic evidence for the differential requirements of platelet integrins in thrombus formation and demonstrate that correct integrin function assessment can be achieved with a combination of diagnostic methods**. Summary and Conclusions.** This set-up can be applied to test in small assay volumes the influence of a variety of stimuli, drugs and plasma factors, such as antibodies, on platelet aggregation. The presented principle stands as a promising user-friendly tool, which allows analysis of platelet aggregation in thrombocytopenic patients or infants, and facilitates studies in platelets obtained from experimental animal models without the need of special devises but a flow cytometer.

0462

NOVEL VON WILLEBRAND GENETIC MUTATION (C.4097T>G) ASSOCIATED WITH VON WILLEBRAND DISEASE TYPE 2M

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We report a previously unreported von Willebrand Factor (vWF) gene mutation associated with an unusual phenotype of von Willebrand Disease (probable vWD Type 2M) characterised by markedly reduced vWF Ristocetin co-factor activity (vWF:RCo) in the presence of normal or high von Willebrand Factor antigen (vWF:Ag), von Willebrand Factor Collagen Binding Acitivity (vWF:CBA), and FVIII:C activity.The proband C (female, 55yrs) and both her daughters (J, 26yrs; L, 28yrs) were referred for investigation of a bleeding disorder. The father of J and L was already known to have mild haemophilia A. C was found to have normal plasma vWF:Ag (120iu/dL; nr 45-160iu/dL) and vWF:CBA (95u/dl; nr 35-140u/dL) but reduced vWF: RCo of 25 iu/dl (nr 54-202iu/dL). Her blood displayed delayed closure times in both collagen/ADP and collagen/epinephrine cartridges in the PFA-100® system. Low resolution Von Willebrand Factor multimeric analysis (Phast system) showed a normal distribution with no deficit in HMW multimers. Her factor VIII:C was 140iu/dl (nr 50-150iu/dL).DNA analysis of the von Willebrand Factor (VWF) gene detected a heterozyogus substitution in exon 28 of her VWF gene (c.4097T>G). This mutation corresponds to the replacement of native phenylalanine at codon 1366 with a cysteine (p.Phe1366Cys). This mutation has not previously been reported but *in silico* analysis strongly supports its being pathogenic and it is in the same region as several other mutations associated with a Type 2M phenotype (E1359K, V1360A, K1362T and F1369I). The associated phenotype suggests that this mutation results in a highly selective effect on vWF binding to its platelet receptor, demonstrated by low ristocetin cofactor activity in the washed-platelet ristocetin co-factor assay without evidence of reduced vWF function in other modalities.Both daughters inherited this mutation, and the associated Type 2M von Willebrand Disease phenotype, from their mother, in addition to inheriting their father's factor FVIII mutation (p.Tyr551Cys). This resulted in J manifesting a mild bleeding disorder with the combined phenotype of Type 2M vWD (vWF:RCo 12.5iu/dL; vWF:Ag 61iu/dL) and mildly-affected carrier of Haemophilia A (FVIII:C 40iu/dL).L. had a FVIII:C level of 64iu/dl (indicating non-affected carrier status for Haemophilia A), vWF:RCo of 19iu/dl, vWF:Ag 79iu/dl. Both sisters also had normal vWF:CBA assays, normal vWF mulimer composition and prolonged closure times in both cartridges of the PFA-100® system.

0463

CONGENITAL FXI DEFICIENCY: EVALUATION OF POST-SURGERY BLEEDING PHENOTYPE AND CORRELATION WITH FXI ACTIVITY (FXI:ACT)

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Background. The bleeding phenotype in FXI deficiency is variable and generally related to surgery/trauma. Moreover, there is a poor correlation between bleeding and baseline FXI:Act. **Aims.** To describe the post-surgery hemorrhagic phenotype of our FXI deficient population and to relate the phenotype with FXI:Act. **Patients and Methods.** Since 1973, we have been following 50 FXI deficient patients: 26 F, 24 M; median age at diagnosis: 34 years, range 1.7-79.6; median follow-up: 1.9 years, range 0.1-36.2; positive family history: 18; median FXI:Act of all patients: 37% (range 0.25-60%; normal values: 70-140); FXI:Act ≤1% in 4 patients, >1≤5% in 10, >5 ≤10 in 2, >10 ≤20 in 3, >20 in 31. **Results.** Eighteen patients experienced bleeding episodes not surgery-related: ecchimoses in 14, hematomas in 7, epistaxes in 11, gastrointestinal hemorrhages in 7, meno-methrorrhagia in 1.Prior to diagnosis, 15 patients underwent 69 major/minor surgeries without prophylaxis: 21 hemorrhages were reported. The median FXI:Act in the bleeders was: 28%, range 0.25-53%. Ten spontaneous deliveries (SD) and 5 caesarian sections (CS) were performed: 1 post-partum hemorrhage occurred (patient FXI:Act 27%). Twenty-eight patients underwent dental surgeries, 7 experienced hemorrhages (bleeders median FXI:Act: 6.9%, range 1-43%). Post-diagnosis, 13 minor/major surgeries were performed, 8/13 with anti-hemorrhagic prophylaxis: plasma in 3 cases, plasma and tranexamic acid in 2 cases, tranexamic acid in 2 cases, desmopressin in 1: no bleedings were reported. Three dental exctractions were performed, 1 prophylactically treated with plasma and tranexamic acid: one bleeding was reported in a patient who self-administered anti-inflammatory drugs (FXI:Act 45%). One CS and 1 SD were performed: desmopressin was used as prophylaxis in 1 case: no bleedings reported. **Conclusions.** We confirm the wide variability in post-surgery bleeding events in FXI deficient patients, not related to the FXI:Act levels. Because of the low correlation between FXI:Act and the phenotype, we highlight the need of laboratory-based prognostic factors for a better management of these patients.

0464

INDIVIDUALIZED REPLACEMENT OF FACTOR VIII ACCORDING TO THE *IN VIVO* RECOVERY IN HEMOPHILIA A PATIENTS DURING SURGERY

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Backgroud and Aims. Although patients' individual pharmacokinetics is considered very important in hemophilia, there have been few reports about the practical result after direct application of individual *in vivo* recovery (IVR) to the surgery. Thus we investigated the correlation between individual IVR and the outcome of each operation. **Methods.** The hemophilia A patients who had blood tests for IVR just before each operations from January 2000 to January 2012 were reviewed. The patients with inhibitor (anti-FVIII) were excluded. The respective surgery cases were divided into 3 groups; A (IVR > 80% of expected), B (IVR < 80% of expected, but they were managed by immediate additional replacement of FVIII), and C (IVR < 80% of expected and the extra supplementations of FVIII were delayed over 3 days). Their each consumption of FVIII and hospitalization periods was analyzed. The Mann-Whitney U test (SPSS ver. 19) with bonferroni adjustment was used to compare inter-group differences.4. ResultsTwenty hemophilia A patients underwent total 27 various operations. Twelve patients were severe (FVIII:C < 1 IU dL^{-1}), 4 patients were moderate (FVIII:C 1-5 IU dL^{-1}), and the remainder were mild (FVIII:C > 5 IU dL^{-1}) hemophilia A. They were all males. Their mean age at the time of surgery was 32 (range from 0 to 69) years old, and mean number of surgery they received was 1.4 (range 1 to 3) times. The total dose of FVIII was respectively 31,693 ± 22,858 (mean ± SD) IU in group A, 53,389 ± 48,778 IU in B, and 166,596 ± 149,327 IU in C. There was no statistical difference between them. The FVIII dose per patient's body weight was respectively 481 ± 195 IU kg^{-1} in group A, 1,311 ± 283 IU kg^{-1} in B, and 3,502 ± 1,529 IU kg^{-1} in C. The mean value was lower in group A than B ($p < 0.001$), and in group B than C ($p = 0.006$). The hospitalization period was respectively 14.5 ± 12.1 days in group A, 13.9 ± 4.0 days in B, and 45.8 ± 15.7 days in C. There was no extension of hospitalization in group B in comparison with A ($p = 1.000$). The mean duration was longer in group C than A and B (respectively, $p = 0.015$ and $p = 0.006$). **Summary and Conclusions**. The medical costs were much more expensive in low IVR groups (B and C) than high IVR group (A) according to our expectation. However, the immediate extra supplementation of FVIII reduced the hospitalization period as well as economized the FVIII consumption per body weight in low IVR group (B). It is very important to check IVR on all occasions just before surgery and supply the FVIII according to the result to minimize the medical cost.

0465

CASE STUDY OF THE USE OF INTRANASAL BEVACIZUMAB TO TREAT RECURRENT EPISTAXIS DUE TO HEREDITARY HAEMORRHAGIC TELANGECTASIA

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Background. Hereditary Haemorrhagic Telangectasia (HHT) is an autosomal dominant condition with a prevalence of approximately 1:10,000 of the population. Severe cases present with recurrent bleeding and may require regular blood transfusions or intravenous iron. Several genetic mutations have been associated with HHT and many of these encode proteins that are involved in the vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-ß) signalling cascades. Furthermore, plasma levels of VEGF and TGF-ß are increased in patients with HHT. Limited published data suggest that interruption of VEGF signalling by Bevacizumab (a humanised monoclonal VEGF inhibitor) may represent a potential therapy for patients with HHT. **Aims.** Our aim was to assess the potential off label use of topical Bevacizumab to treat patients with severe epistaxis due to HHT. **Methods.** We treated a 65 year old male patient with a life-long history of severe epistaxis due to HHT. He was dependent upon regular iron infusions and had also required frequent blood transfusions as well as pulsed dye laser therapy given monthly for over 20 years. A history of pulmonary embolism and intracoronary stents precluded use of antifibrinolytic drugs or intravenous Bevacizumab.After informed consent was obtained, we injected a total dose of 100 mg of undiluted Bevacizumab via an intranasal atomization device given in 100 µL volumes to each nostril every five minutes until completion of the 4 mL ampoule. This was repeated on a monthly basis for three months. The severity of his symptoms were assessed using a normalised Epistaxis Severity Score (ESS) to give a value out of 10. **Results.** After three months, subjectively, his quality of life had improved remarkably. His average daily duration of epistaxis reduced from 17 to less than 2 minutes. Objectively, his ESS has reduced from 10 to 3, and his need for pulsed dye laser therapy has diminished. **Summary and Conclusions.** Bevacizumab can be sprayed intranasally to treat severe recurrent epistaxis due to HHT. We are planning to investigate further the potential of this therapeutic approach in further patients with severe epistaxis due to HHT. We anticipate that, in addition to the above therapeutic benefits, patients will require fewer blood transfusions and less intravenous iron support. It is, therefore, likely that there will also be a significant economic advantage to this novel treatment.

0466

PLATELET FUNCTION DIAGNOSTIC LABORATORY INVESTIGATION IN THALASSAEMIC PATIENTS

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Platelet function disorders constitute a rare cause of symptomatic bleeding. Haemostatic disorders are reported in thalassaemic patients. The aim of this study was the platelet function investigation in thalassaemics and the detection of any relation with the chelation treatment. The platelet function investigation consisted of the aggregation testing in platelet rich plasma with light transmission aggregometry (LTG) by the use of 5 agonists (arachidonic acid, ADP, adrenaline, collagen, ristocetin), and the global test of haemostasis by PFA-100 (Dade Behring) whose principle is the *in vitro* platelet plug formation under sheer stress with either collagen and epinephrine or collagen and ADP as agonists. The P2Y12 receptor was also tested.39 thalassaemic patients (24 males, 15 females, aged 5 to 45 years) were included. Patients were on chelation treatment with either deferasirox (18 patients), or deferiprone, desferioxamine or both (21 patients). Eight were splenectomised and six received aspirin as prophylactic treatment, seven patients had a liver biopsy. No haemostatic cover was used and no untoward bleeding was reported. One patient had experienced a peptic ulcer bleeding. Four cases with chronic viral hepatitis were included.RESULTS66% (26) of the patients showed reduced LTG with adrenaline and 20% (8) showed reduced LTG to two or more of the agonists used. 34 patients were tested with PFA-100 and was prolonged in 73% (25) of them. 43,5% (17 patients) had both a defect in platelet aggregation and a prolonged PFA-100 test, while 10,2% (4) had abnormal LTG and normal PFA-100 time. 20,5% (8) patients had normal aggregation and abnormal PFA-100 test, while 10,2% (4) had both normal aggregation and PFA-100 time. Three patients with chronic hepatitis had reduced LTG and one was found normal. The patients that had undergone liver biopsies had abnormal LTGs and 4 had prolonged PFA-100. Among the patients that had undergone a splenectomy, we found five of them to have abnormal LTG and prolonged PFA-100, while two of them had normal tests. The patient with GI hemorrhage had abnormal LTG and PFA-100. We found no correlation with either chelation regimen and LTG or PFA-100.Our patients' results are indicative of a platelet defect similar to disorders of platelet secretion and signal transduction. The clinical manifestations though were few. The defective LTG can be explained by the release of ADP from the haemolysed red blood cells, which leads to defective platelet aggregation and secondly the presence of two platelet populations in the circulation of the patients. The more active platelets present as circulating aggregates and are not detected by *in vitro* study and the less active are poorly aggregable. An alternative explanation is the *in vitro* effect.In literature and clinical practice there is a lack of consensus about the best diagnostic tests concerning platelet function. In thalassaemics the reduced LTG and the prolonged PFA-100 closure time must not be overestimated due to the limited clinical manifestations. The administration of salicylates in splenectomised thalassaemics should not be ignored. Nevertheless, bleeding can occur in patients with platelet defects of the above type.

0467

ACQUIRED HAEMOPHILIA A-RETROSPECTIVE ANALYSIS OF 46 CASES FROM A SINGLE CHINESE HAEMOPHILIA CENTER

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Background. Acquired hemophilia A (AHA) is a rare disorder caused by the autoantibody directed against factor VIII in patients without previous history of a bleeding disorder. Severe bleeding occurs in a majority of patients with a high rate of mortality. The patients of AHA usually present to clinicians without experience of this disease. As a result, the diagnosis and proper treatment is often delayed. **Aims and Methods.** We retrospectively analyzed the characteristics and outcomes of 46 patients with AHA from 1994 to2011 in our center. **Results.** The median age was 53 years ranging from 14 to 78 years.The major peak of age was 60-70 years 16 of 46, 35%, the second frequency peak was 20-30 years 9 of 46, 20%. The associated disease was observed in 7 (16%) patients. Nine patients had medical conditions that were unlikely to be related to acquired inhibitors. The symptoms included ecchymosis (44 of 46, 96%), muscle or soft tissue haematoma(33 of 46,77%), hematuria (12 of 46,28%), mucous bleeding (10 of 46, 21%), hemarthrosis (7 of 46, 16%), intracranial bleeding (2 of 46, 5%). Twenty-four patients with acute bleeding episode were treated with prothrombin complex concentrate (PCC) at a relative low dose of 30-50U/kg· d and achieved good outcomes without adverse reaction. Corticosteroids alone or combined with cyclophosphamide were used as first-line therapy to eradicate the inhibitors. In 37 evaluable patients, 33 (89%) cases achieved complete remission (CR). Four cases died of hemorrhage and 2 of them had neutropenia-associated infections which conversely aggravated haemorrhage. The response time was not related to FVIII:C and inhibitor titre. Two patients relapsed after 4.5 months and 1 year respectively, but they achieved second CR after the second cycle of therapy. **Conclusions.** This study summarizes the experience about AHA from a single Chinese hemophilia center where expensive bypassing agents such as recombinant activated factor Ⅶ and activated prothrombin complex concentrate are not available . We find that PCC is effective and safe to control acute bleeding. Corticosteroids alone or combined with cyclophosphamide used as first-line therapy achieved good outcomes with CR rate of 89%. The comorbidities and side-effect of immunosuppressant in patients probablely have adverse impact on the prognosis.

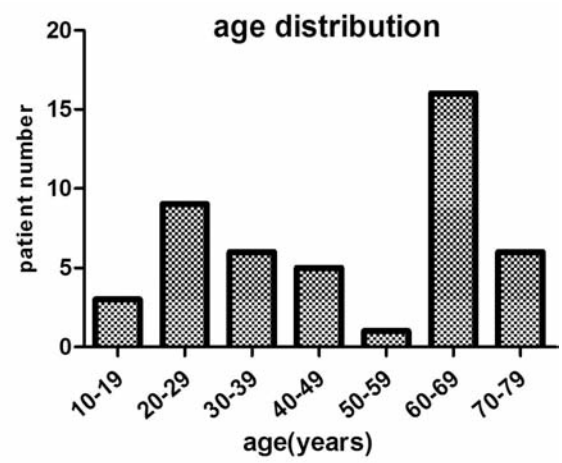

Figure 1. Age distribution.

0468

A SINGLE CENTRE EXPERIENCE OF INHIBITOR ERADICATION THERAPY IN SEVERE HAEMOPHILIA A

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Background. One of the most serious complications of haemophilia A is the development of factor VIII (FVIII) inhibitors. Immune tolerance induction (ITI) protocols use FVIII at varying doses with the addition of other immunomodulatory therapies in non-responders. This is a 20 year single centre experience of inhibitor eradication therapy in severe haemophilia A. **Methods.** We reviewed the heath care records of all patients with severe haemophilia A (FVIII: C <0.01 iu/ml) who had developed a factor VIII inhibitor between 1990 and 2011. We identified 22 patients. Patients were excluded from the study if clinical details were inadequate or if the inhibitor was transient. All patients underwent ITI with recombinant FVIII (rFVIII) ± immunomodulatory therapy. Immune tolerance was defined as 3 consecutive negative monthly inhibitor screens with FVIII recovery greater than 66%.ResultsWe report on 14 children (ten unrelated and two brother pairs) with severe haemophilia A and FVIII inhibitor. Age at inhibitor detection ranged from 0.9 months to 79 months and number of exposure days varied from 5 to 182 (Table 1). Circumstance of inhibitor development is shown in Table 1. Time from first exposure to inhibitor diagnosis was 0.5 to 79.1 months. 50% had high responding inhibitors [>10 Bethesda units (BU)] with peak inhibitor titre ranging from 10 to 712 BU. All patients had received rFVIII. ITI was started when the inhibitor titre was <10 BU in 11 patients. Three patients started ITI at inhibitor titres of 17, 26 and 256 BU respectively. ITI rFVIII schedules varied from low dose (50 IU/kg 3 times weekly) to high dose (100 IU/ kg twice daily). Three patients with high inhibitor levels (141, 276 and 712 BU) also received immunosuppressive therapies; Rituximab, vincristine, prednisolone and mycophenolate mofetil. All 14 patients had a central venous device (CVC) inserted. Eight had CVC related infection(s), necessitating CVC removal and subsequent replacement in seven of the eight patients. Breakthrough bleeding was treated with rFVIIa. Immune tolerance was achieved in all patients. Time to elimination of inhibitors ranged from 1.2-48.3 months (median of 9 months). All patients are now on rFVIII prophylaxis.ConclusionDespite the variability of the patient characteristics and the ITI schedules, all patients were successfully tolerised with rVIII.

Table 1. Summary of inhibitor development. Patients 1, 4 and 9 received other immunomodulatory therapies. Patient 4 had 2 relapse needing further ITI.

Patient	Age (months)	Highest inhibitor (BU)	Inhibitor at ITI start (BU)	Total exposure days	Circumstance of inhibitor formation	ITI duration (months)
1*	22.7	141	N/A	13	Joint bleed	32.4
2	23.6	6	6	12	GI bleed	11.0
3	23.3	17	0.7	7	Head injury	7.0
4*+	6.5	276	256	10	Joint bleed	48.3
5	24.4	26	26	11	Prophylaxis	8.3
6	13.1	10	1	7	Port insertion	1.9
7	17.6	1.5	6	8	Prophylaxis	9.7
8	79.1	18	3	182	Prophylaxis	2.5
9*	10.1	712	17	6	Joint bleed	14.0
10	0.9	3.5	3.5	14	Cephal-haematoma	11.0
11	22.2	5.8	5.8	10	Psoas bleed	1.2
12	12.3	1.1	1.1	14	Prophylaxis	13.0
13	18.6	2.6	Borderline	5	Trauma	7.8
14	8.4	3.9	3.9	5	Joint bleed	1.8

0469

MANAGEMENT OF PATIENTS WITH HHT DURING PREGNANCY

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Background. Hereditary Hemorrhagic Telangiectasia (HHT) is a genetic disorder of the blood vessels, which affects approximately 1 in 5000 people. Pregnant women with HHT should be considered 'high risk'. They should regularly attend multidisciplinary (Consultant Haematologist, Consultant Obstetrician, Consultant Anesthetist and midwives) high risk clinics. At these clinics the women will be advised on the pregnancy-associated risks. The women should highlight if and when they experience any haemoptysis or sudden severe dyspnoea, which can lead to immediate hospital admission. The outcome for any high-risk pregnancy is better when the high-risk multidisciplinary team is informed and have a plan of care in place. **Aims.** We highlight the management of 3 women (five pregnancies) with significant issues pertaining to the management of HHT in pregnancy. **Methods.** PAVMsIdeally women planning a pregnancy should have pre-pregnancy screening of PAVMs and treated accordingly. If there was no recent screening of PAVMs prior to conception then regular screening of PAVMs at the high-risk clinic and treatment of same during the pregnancy.MRIsA spinal MRI must be carried out during pregnancy ideally ear ly in the pregnancy to ensure there are no spinal AVMs and also to allow for regional anesthesia.A cerebral MRI must be carried out on women experiencing cerebral symptoms or a strong family history of cerebral hemorrhage, or cerebral symptoms. Delivery. All women must receive prophylactic antibiotics during her labour. Prolonged second stage of labour should be avoided especially in women where cerebral AVMs have not been excludedIf general anesthesia is required, a modified induction regimen using opiates should be administered. Postnatal. Refer women and baby back to the nearest HHT centre and ensure family screening where not previously not done. **Summary and Conclusions.** Most cases where HHT is known in pregnancy the outcome is favorable as the pregnancy proceeds normally. Due to the complications associated with HHT these pregnancies should be considered high risk**.**

0470

FREQUENCY OF BLEEDING DISORDERS IN WOMEN PRESENTING WITH MENORRHAGIA IN THE NORTH OF IRAN

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Background. Menorrhagia is a common presentation of bleeding disorders, especially VWD in women. **Aims.** We decided to determine the frequency of these disorders in women with menstruation problems. **Methods.** 208 patients in reproductive age with menorrhagia were investigated for bleeding disorders in two steps. Step one includes CBC, PT, PTT and BT tests which were performed for all patients; patients who had an abnormality in step one, the second step tests including VWF: A;VWF: RCo, factors level, RIPA and platelet aggregometry were performed. **Results.** Of 208 patients who were investigated for bleeding disorders, 53 patients (25%) had abnormalities in coagulation tests or platelet counts. Frequencies of bleeding were as follows: VWD, 14(6.73%); thrombocytopenia,13(6.25%); factor II deficiency , 2(0.96%); factor V,1(0.48%); factor VII,3(1.44%); factor VIII,2(0.96%); Factor XI,4(1.92%); factor XII,4(1.92%); Bernard Soulier,2(0.96%). Furthermore, we found that 18 patients (8.65%) had abnormal PT, PTT or BT with no definite diagnosis. **Conclusions.** In this study, the most common bleeding disorder was VWD followed by thrombocytopenia. Although other bleeding disorders are rare, in our study a number of them were found. We recommend that, every woman with menorrhagia should be offered the above coagulation tests.

0471

SCREENING BLEEDING DISORDERS IN ADOLESCENTS AND YOUNG WOMEN WITH MENORRHAGIA

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Background. Chronic menorrhagia causes anemia and impairment of life quality. **Aims.** To screen bleeding disorders in adolescents and young women with menorrhagia. **Methods.** The study was performed prospectively by the pediatric hematologists. A form including demographic characteristics of the patients, bleedings other than menorrhagia, familial bleeding history and characteristics of the menorrhagia and impairment of the life quality due to menorrhagia was filled by the researcher during face-to- face interview with the patient. Pictorial blood assessment chart was also delivered for evaluation of blood loss. All the patients underwent pelvic examination and pelvic USG by the gynecologists. Whole blood count, peripheral blood smear, blood group, serum transaminases, urea, creatinine, ferritin, PFA-100, PT, aPTT, INR, TT, fibrinogen, VWF:Ag, VWF:RCo, FVIII, platelet aggregation assays were performed. Platelet aggregations were studied by lumiaggregometer. **Results.** Sixty patients completed the study. Mean age was 20.68±10.34 (10-48) years and 65% (n=39) of the patients were younger than 18 years. In 18 (46%) of the adolescents menorrhagia subsided spontaneously. In 20% (n=12) of the patients, a bleeding disorder was detected (4 Von Willebrand disease, 3 Bernard-Soulier syndrome, 2 Glanzmann thrombastenia, 2 immune thrombocytopenic purpura, 1 congenital factor VII deficiency). **Summary and Conclusions.** In patients with menorrhagia at least complete blood count, peripheral smear, aPTT, PT, VWF:Ag, VWF:RCo, and fibrinogen assays must be performed. When there is history of nose and gum bleeding, platelet function assay by lumiagregometer must also be performed. In nearly half of the adolescents, menorrhagia is dysfunctional and transient. Detailed coagulation assays can be postponed in adolescents if bleeding history other than menorrhagia and/or family history of bleeding and/or parental consanguinity is absent. All subjects with menorrhagia must be consulted with gynecologists and hematologists.

0472

THROMBIN GENERATION AND MICROPARTICLE-ASSOCIATED PROCOAGULANT ACTIVITY AS NEW TOOLS TO EVALUATE THE HEMOSTATIC PROPERTIES OF PLATELET CONCENTRATES

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Introduction. Thrombin generation (TG) and platelet microparticles procoagulant activity (MP-PCA) are two emerging assays utilized to characterize the hemostatic profile of plasma samples. **Aims of the study.** In this study we evaluate: 1) the applicability of TG and MP-PCA assays in assessing the procoagulant properties of platelet concentrates (PC) prepared for transfusion therapy; and 2. whether these assays are sensitive to the methodology used for PC preparation. **Methods.** PC were prepared from pooled buffy-coat of overnight-stored whole blood from healthy donors at the Immunohematology and Transfusion Medicine Dept of Bergamo (Italy). 10 PC were prepared with the Fenwal System (FS) and 4 with Terumo TACSI System (TTS). PC samples were collected the day of preparation (D0) and after 3 days of storage (D3) at 22°C, and then processed to isolate supernatants of PC (S-PC) by two serial centrifugations. S-PC were tested for their TG potential by the calibrated automated thrombogram (CAT) assay employing 1 and 5 pM TF concentrations (both with 4 uM phospholipids), and for MP-PCA by the P-PPL/1 kit (all reagent by Stago). Results of TG are expressed as lag-time, peak, area under the curve (ETP), and time-to-peak (ttPeak). Results of MP-PCA are expressed in seconds (sec). **Results.** At D0, TG of S-PC was directly proportional to the concentration of TF used as trigger, and similar to that generated in normal pool plasma (NPP). Lag-time of S-PC derived from PC prepared with TTS was significantly shorter compared to those prepared with FS ($p<0.05$), being the other TG parameters not different. Lag-time (+43%) and ttpeak (+24%) of S-PC collected after 3 days of storage were significantly ($p<0.05$) increased compared to D0, independently of the type of PC preparation. Differently, we could observe a significant decrease of ETP in S-PC prepared with TTS (1478±238 *vs* 1058±216 nM*min; $p<0.05$), but not in S-PC prepared with FS (1501±240 *vs* 1440±216 nM*min). At baseline, MP-PCA in S-PC (80.34±9.21 sec) was similar to that found in NPP (82.5±10.01 sec; p=ns). The analysis according to method of PC preparation did not show any significant difference in MP-PCA at baseline. The MP-PCA of S-PC collected at D3 was significantly higher compared to D0 ($p<0.05$), as this time point the S-PC prepared with TTS (TTS: 84±6.5; FS: 78±10.01 sec) showed the highest procoagulant potential. **Conclusions.** Our data support the validity of CAT and P-PPL1 assays in the assessment of procoagulant activity of PC. The increased MP-PCA observed in samples collected after 3 days of storage reveals the occurrence of platelet activation/apoptosis, particularly in S-PC prepared with TTS. These results provide background for further evaluation of the usefulness of these new tests to assess the quality of PC.

Thrombosis and vascular biology 1

0473

THE EGFP-EGF1-PLGA NANOPARTICLE MEDIATED THE TARGETED DILEVERY OF SIRNA FOR THE CEREBRAL THROMBUS THERAPY IN VITRO.

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Background. It was reported that the fusion protein EGFP-EGF1 ,which had the specific TF binding capacity,could improve the cerebral thrombus targeting property of PEG-PLA nanoparticles. **Aims.** The main objective of our study was to developed the EGFP-EGF1-conjunated PLGA nanoparticles(ENP) as a new targeted dilevery of the TF-specific siRNA for the gene therapy of cerebral thrombosis *in vitro*. **Methods.** Firstly,we established a cerebral thrombus model *in vitro*,which the brain capillary endothelial cells (BCECs)were treated with TNFα.Then the treated cells were transfected with the siRNA/ENP complex.The gene silencing of tissue factor(TF) and the TF activity were measured.And the cytotoxicity of the nanocarrier was also determined. **Results.** After transfection,the total RNA were isolated from the cells for real-time PCR and the efficiency of the TF mRNA downregulation was 1.64 folds compared with siRNA/NP.The TF protein expression,determined by the western-blotting and flow cytometry ,decreased 58.5% and 80.41% of the treated BCECs respectively,while the efficiency of downregulation were 1.41 folds and 1.34 folds than siRNA/NP.Moreover,the relative TF activity in siRNA/ENP group was lowest and was 1.5 folds than the siRNA/NP group.With the dose of the nanocarrier increasing,there were almost nontoxity to BCECs.The TF expression could be inhibited by siRNA/ENP complex in both quantity and quality in treated BCECs and the gene silencing efficiency was better than siRNA/NP complex. Conclusion:The EGFP-EGF1-PLGA nanoparticle provided a targeted delivery of TF-specific siRNA and the siRNA/ENP complex was effective for the gene therapy of cerebral thrombosis *in vitro*.

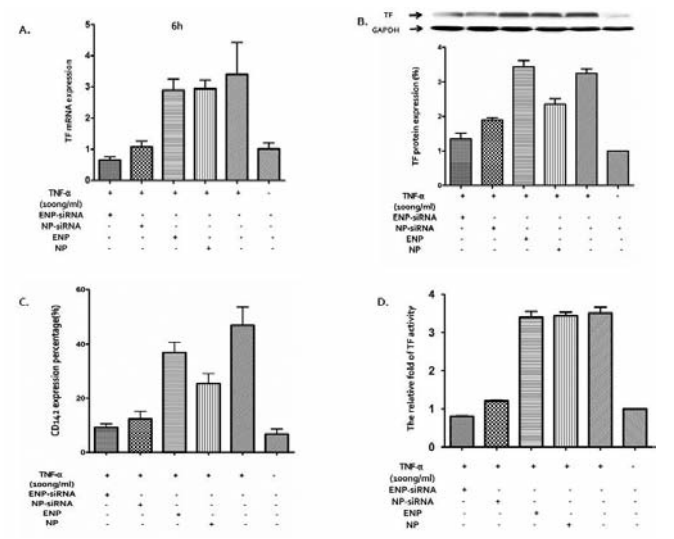

Figure 1. Gene silencing.

0474

EFFICACY OF LOW MOLECULAR WEIGHT HEPARIN IN PREVENTING PREGNANCY-RELATED RECURRENT VENOUS THROMBOEMBOLISM.

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Background: Women with a history of venous thromboembolism (VTE) have an increased risk of recurrence in subsequent pregnancies, so that prophylaxis with low molecular weight heparin (LMWH) is warranted in most of them during pregnancy and puerperium. However, efficacy of LMWH has been debated, suggesting to be dose-dependent. **Aims.** To assess the efficacy of LMWH among pregnant women with a history of VTE. **Patients and Methods.** We analyzed a cohort of 627 women who had had deep vein thrombosis and/or pulmonary embolism before 45 years of age and were referred to our Center; 84 of them had been pregnant at least once after VTE and were considered suitable for the study. The presence of inherited thrombophilia and/or antiphospholipids was not a criterion of exclusion. We stratified women according to use of prophylaxis with LMWH during pregnancy and puerperium (defined as 6 weeks after delivery at >16 weeks' gestation). During the first pregnancy after VTE, 40 women (18 with thrombophilia) received LMWH and 44 (19 with thrombophilia) did not. Six women were at highest risk (two VTE events before pregnancy) and received LMWH in 5 cases. All the unprophylaxed pregnancies and 6 prophylaxed pregnancies occurred before referral to our Center, whereas 85% of the prophylaxed pregnancies occurred after referral and were prospectively followed. LMWH was b.w.-adjusted (nadroparin 3800 or 5700 IU o.d. and enoxaparin 4000 or 6000 IU o.d. for b.w. < 70 kg or > 70 kg, respectively). Only 2 women received nadroparin 2850 IU o.d. To assess the ante-partum efficacy of LMWH we compared the cumulative probability of recurrence according to the period of pregnancy between treated and untreated women by the Kaplan-Meier method. In order to ensure the independence of the observations, we avoided multiple correlated outcome events in women with multiple pregnancies after VTE considering only the first pregnancy after VTE. The rate of VTE during puerperium was also recorded. **Results.** During pregnancy there were 6 DVTs (13.6%, 95%CI 6.4-26.7) among the untreated women and 1 DVT (2.5%, 95%CI 0.4-12.8) among the treated women. The hazard ratio of recurrent DVT during pregnancy was 0.14 (95%CI 0.04-0.91, p=0.03) among the women receiving LMWH in respect to those without treatment (Figure). Fetal loss before the 16th week of gestation occurred in 10 women without LMWH and in 1 woman receiving LMWH (p=0.007); there were 3 DVTs during 34 puerperium periods without LMWH (8.8%, 95%CI 3.0-22.9) and 2 DVTs during 39 puerperium periods with LMWH (5.1%, 95%CI 1.4-16.8, p=0.65). None of women at highest risk or receiving nadroparin 2850 IU o.d. had DVT. Among the untreated women, heterozygous factor V Leiden was present in 2 with ante-partum DVT and in 2 with post-partum DVT (odds ratio for overall pregnancy-related recurrent DVT associated with thrombophilia 1.06, 95% 0.24-4.66). **Conclusions.** In women with a history of DVT the use of b.w.-adjusted LMWH significantly reduces the rate of ante-partum recurrent DVT by 86%. The rate of puerperium-related recurrent DVT does not seem to be significantly reduced by prophylactic doses of LMWH.

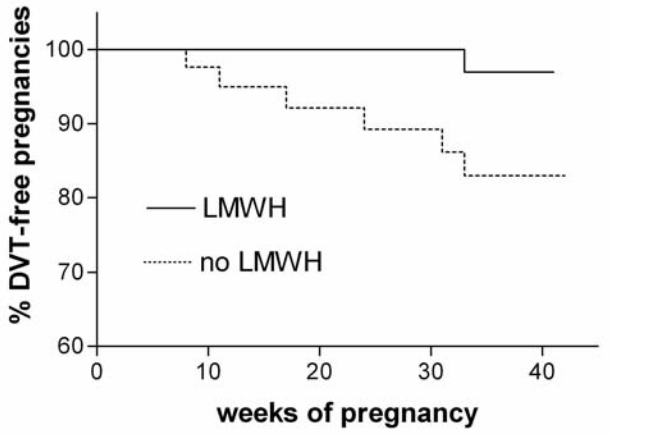

Figure 1.

0475

THE RISK OF FETAL LOSS IS INCREASED IN WOMEN WITH INHERITED THROMBOPHILIA AND FAMILY HISTORY OF OBSTETRIC COMPLICATIONS.

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Background. Several studies have demonstrated an association between inherited thrombophilia and obstetric complications (OC), in particular recurrent fetal loss (greater than 2) and unexplained intrauterine fetal death after the 20th gestational week. However, the strength of association is weak and it is uncertain whether women with inherited thrombophilia are more prone to OC than non-carriers. **Aims.** To assess the risk of OC among the carrier women with inherited thrombophilia. **Methods.** We carried out a family study and we analyzed the clinical history of 415 women recruited from a large family cohort

of 1,720 relatives of 563 probands with inherited thrombophilia. The inclusion criteria were a) to be a relative of a proband diagnosed as carrier of inherited thrombophilia after venous thromboembolism (VTE) or OC; b) to have been genotyped for inherited thrombophilia; c) to have been pregnant at least once. The presence of antiphospholipids was a criterion of exclusion. **Results.** The two study groups consisted of 93 relatives (54 carriers) of a proband with OC, and 322 relatives (187 carriers) of a proband with VTE. Out of 241 carriers, 13 had antithrombin, protein C or S deficiency, and the remaining ones had factor V Leiden and/or prothrombin G20210A. Overall, we recorded 1,038 pregnancies. If the proband had OC, the rate of fetal loss was 21.4% among carriers (32 of 149 pregnancies) and 15.3% among non-carriers (15 of 98 pregnancies); if the proband had VTE, the rate of fetal loss was 14.2% among carriers (64 of 450 pregnancies) and 12.6% among non-carriers (43 of 341 pregnancies). The carriers with a history of fetal loss were 22 among the relatives of a proband with OC (40.7% of the carriers), and 47 among the relatives of a proband with VTE (25.1%); the non-carriers with a history of fetal loss were 9 and 34, respectively. In women with thrombophilia in comparison with non-carrier women, the odds ratio (OR) of having at least one fetal loss was 2.29 (95%CI 0.91-5.76) among the relatives of a proband with OC and 0.99 (95%CI 0.59-1.66) among the relatives of a proband with VTE. The risk for fetal loss was double in the carriers identified because a history of OC in the proband in respect to the carriers identified because a history of VTE in the proband (OR 2.04, 95%CI 1.08-3.86). Moreover, the risk of VTE was 3.78-fold increased (95%CI 1.40-10.23) among carriers who were relatives of a proband with VTE (12.2%) in respect to non-carriers (3.7%); on the opposite, the rate of VTE was not different between carriers who were relatives of a proband with OC and non-carriers (3.7% versus no events, p=0.51). **Conclusions.** In women with inherited thrombophilia and family history of OC the risk of fetal loss is increased. Whether this information could be applied in tailoring pharmacological prophylaxis in their pregnacies should be investigated in proper studies. Finally, we confirmed that the risk of VTE among family members with inherited thrombophilia is dependent on the clinical phenotype of the proband (VTE or OC).

0476

INHIBITING ANGIOGENESIS ACTIVITIES OF QUERCETIN HYDRATE IN ZEBRAFISH

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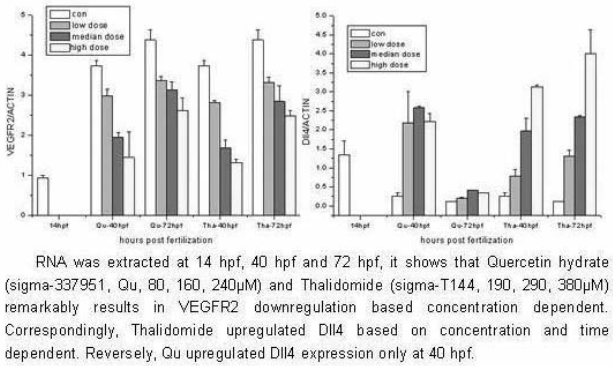
Background. Several molecules isolated from Chinese herb can inhibit the growth of cancer cells with an ability of antiangiogenic. Angiogenic homeostasis is maintained by a balance between vascular endothelial growth factor (VEGF) and Notch signalling in endothelial cells (ECs), which are closely related to tumor diameter, clinical stage, histological grade and lymph node metastasis. Quercetin (Qu) is considered an excellent “chemopreventer”, but the underlying mechanism still is unclear. **Aims.** To investigate the antiangiogenic effects of Quercetin hydrate (sigma-337951, Qu) *in vivo*. **Methods.** Tg(fli1:EGFP) transgenic zebrafish embryos were treated with different concentrations of Qu (80, 160, 240μM), 0.1% DMSO as vehicle control and different concentrations of Thalidomide(sigma-T144, 190, 290, 380 μM) as positive control from 14 hours post fertilization (hpf) to 72 hpf. Thalidomide has a significant antiangiogenic effect against VEGF-induced neovasclar growth. Taking the advantage of the transparent characteristics of the zebrafish, we dynamically observed green intersegmental vessels (ISVs) real time by laser confocal microscope imaging technology. Kinds of transcription factors in Dll4/Notch (Delta-like 4 is the only ligand of Notch expressing in endothelium) and VEGF(R) signaling pathway were evaluated using quantitative real-time PCR (qRT-PCR) and Western blotting. **Results.** When zebrafish were analyzed at 72 hpf, a time point where all ISVs in the vehicle control group have fully extended to form the dorsal longitudinal anastomotic vessels (DLAVs), Qu-treated and Thalidomide-treated groups were found to result in significant reductions in the number of complete ISVs and angiogenic sprouts compared with the vehicle control group, with a greatest reduction in embryos treated with high concentration. These results demonstrate that Qu serves as an inhibitor of ISVs angiogenesis in zebrafish embryos. We extracted RNA and protein from these embryos at 14 hpf, 40 hpf and 72 hpf, respectively, found that Qu and Thalidomide remarkably results in VEGFR2 (VEGF Receptor 2) downregulation based on concentration dependent. Correspondingly, Thalidomide upregulated Dll4 based on concentration and time dependent. Reversely, Qu upregulated Dll4 expression only at 40 hpf VEGF. And there were no significant change of VEGF, VEGFR1, Notch1, Delta like I□3(ligand of Notch) detection.**Summary.** Zebrafish represents a powerful model system for chemical discovery and gene targeting in tumor angiogenesis. Our results indicate that significant inhibition of zebrafish embryos’ angiogenesis was caused by doses under the minimum lethal concentration of Quercetin treatment at 14 hpf (when the ISVs begin to bud) and mainly via inhibition of VEGFR2-mediated signaling. It has an instantaneous effection on Dll4/ Notch signaling pathway. No obvious side effects were recorded. We present a comprehensive framework of study using fluorescence microscopy, transcriptomics and qPCR to demonstrate the proangiogenic effects of Quercetin *in vivo*. The data have elucidated the connection between morphological observations and genomic evidence, indicating the potential roles of several key signaling pathways in angiogenesis.



RNA was extracted at 14 hpf, 40 hpf and 72 hpf, it shows that Quercetin hydrate (sigma-337951, Qu, 80, 160, 240μM) and Thalidomide (sigma-T144, 190, 290, 380μM) remarkably results in VEGFR2 downregulation based concentration dependent. Correspondingly, Thalidomide upregulated Dll4 based on concentration and time dependent. Reversely, Qu upregulated Dll4 expression only at 40 hpf.

Figure 1. The outcome of qRT-PCR.

0477

HIGH ON-TREATMENT PLATELET REACTIVITY: RISK FACTORS AND 3-YEARS OUTCOMES IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background. High on-treatment platelet reactivity (HTPR) is expected to be a negative prognostic factor in patients with stable coronary artery disease. But in patients with acute coronary syndrome the data about prognostic value and association with risk factors are lacking.Objectives: The aim of the study was to assess the relationship between HTPR, potential risk factors and with three-year mortality in patients with acute coronary syndromes. **Patients and Methods.** We performed a prospective cohort study of 198 patients with acute coronary syndrome. In these patients the response to aspirin and clopidogrel was assessed by the impedance aggregometry. According to the response to antiplatelet treatment patients were divided in groups with sufficient response, dual poor responsiveness (DPR), poor responsiveness to aspirin (PRA) and poor responsiveness to clopidogrel (PRC). Simultaneously the age, gender, left ventricle ejection fraction, presence of heart failure, diabetes mellitus and smoking habit was recorded. After three years, the myocardial reinfarction incidence and overall mortality were assessed. **Results.** Poor response to antiplatelet treatment was significantly more frequent in patients in NYHA III and IV class (HR 8.35, 95 % CI 3.7-18.8, p<0.0001 for DPR, HR 3.47 95 % CI 1.95-5.57, p<0.0001 for PRA, HR 4.34, 95 % CI 2.58 - 6.51, p<0.0001 for PRC) and in patients with left ventricle systolic dysfunction (HR 1.86, 95 % CI 3.29-1.34, p<0.05 for PRA). Three-year mortality was significantly higher in all groups of patients with HTPR compared with patients with sufficient response to antiplatelet treatment: in patients with poor responsiveness to aspirin 31.7 % vs. 11.5 %, p <0.01, with poor responsiveness to clopidogrel 33.3 % vs. 10.9 %, p<0.001 and with poor response to both aspirin and clopidogrel 40.9 % vs. 12.5 %, p<0.001. Risk of repeated myocardial infarction was increased as well (HR 4.0, 95 % CI 1.25-11.5, p<0.05 for DPR, HR 4.37, 95 % CI 1.51-12.77, p<0.01 for PRA, HR 3.25, 95 % CI 1.11-9.36, p<0.05 for PRC). **Conclusions.** Poor response to aspirin, clopidogrel or both aspirin and clopidogrel are strong negative risk factors of death and repeated myocardial infarction in patients with acute coronary syndrome. We found a heart failure and left ventricle systolic dysfunction to be risk factors of HTPR development.

0478

AUTOPSY PROVEN FATAL PULMONARY EMBOLISM CAUSED BY HOSPITAL ACQUIRED THROMBOSIS: AN EXEMPLAR CENTRE EXPERIENCE

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Background. Pulmonary Embolism (PE) is an important cause of death in hospitalised patients but significantly under-reported due to non-specific presentations and falling autopsy rates. An estimated 250,000 deaths are reported to be caused by preventable Hospital Acquired Thrombosis (HAT) in England. HAT includes venous thromboembolism (VTE) which occurs during inpatient stay of more than 3 days and within 90 days of discharge.AimTo determine the number of fatal PE associated with HAT and to use this data to help inform outcome metrics associated with venous thromboembolism (VTE) prevention initiatives within the a teaching hospital and DoH Exemplar centre.MethodA retrospective review of the autopsy reports was carried out over a 12 month period in 2011. The case notes were identified where PE was the cause of death and there had been an in-patient stay within 90 days prior to death. The latter detail was obtained from the PM reports and patients notes. Three notes from the HAT group were unobtainable.Results1009 autopsies were performed over 12 months (autopsy rate = 44%). Fatal PE was identified in 61 cases (6.1%). Of these deaths, 20 (33%) were associated with HAT according to detail from PM reports and the notes were reviewed: Five deaths (8.2%) occurred as in-patient, 6 (9.8%) within 30 days of discharge and 6 (9.8%) within 90 days of discharge. Median Age was 78 years (range 52-98) with a male to female ratio of 1:3. Median duration of hospital stay was 15 days (range 5-120). Five patients had advanced cancer, 4 patients underwent major operations, 3 patients had sepsis and 2 had significant dehydration. VTE risk assessment according to NICE guideline was carried out in 12/17cases where notes were available (71%). Thromboprophylaxis (TP) was given in the form of low molecular weight heparin (enoxaparin®) to 15/17 (88%). However of the 15 patients who received enoxaparin®, 3 (20%) had unexplained missed doses, making a total of 5 patients who experienced potentially preventable fatal PE. SummaryWhen this data is compared to the autopsy analysis of the previous year (2010) where 19 deaths (26%) were identified as being HAT events, there is a 7% increase. Whereas in 2009, 35 deaths (41%) were recorded as HAT events. In 2010, there were no cases of preventable death from fatal PE and one from 2009. However, the previous data did not include missed doses and therefore the comparison is not accurate. Since the introduction of NICE VTE Clinical Guideline 92, in January 2010, there has been an emphasis on thorough VTE risk assessment and appropriate TP on admission to hospital, regular auditing by a designated VTE prevention team and real-time feedback to clinicians may have played a role in a reduction of fatal PE due to HAT over the last 24 months. There has also been a focus on other measures to reduce VTE events such as early mobilisation, adequate hydration, regional anaesthesia and advances in surgical technique.

0479

RECURRENT PEDIATRIC THROMBOSIS: INFLUENCE AND UNDERLYING OR COEXISTING FACTORS

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Background. Despite advances in our understanding of the pathogenesis and risk factors of pediatric thrombosis, the experience of recurrent venous thrombosis (rVTE) in children is still limited. It is a well-identified issue that after a first episode of thrombosis, one has the risk of recurrence lifelong. Some factors may constitute the risks of rVTE such as multiple prothrombotic mutations, persistently elevated factor VIII and/or d-dimer levels, positive family history of rVTE. **Aims.** To evaluate the underlying diseases, thrombus localizations and other risk factors of the pediatric patients with recurrent thrombosis in order to obtain a sense of early awareness. **Materials and Methods.** We retrospectively evaluated both inherited & acquired thrombophilic risk factors in children with recurrent thrombosis who were diagnosed and treated at Pediatric Hematology Division, Hacettepe Medical Faculty, Ankara, Turkey. The confirmation of a newly developed thrombosis by classic imaging modalities seven days after the first episode was defined as recurrence. Both congenital & acquired risk factors responsible for the recurrence and treatment modalities were analyzed in detail. **Results.** Of 569 children with thrombosis, 32 (5.6%) presented with rVTE. The median age at the first presentation (11 females, 34.4%) was 132 months. Twenty-nine (90.6 %) had an underlying chronic disorder of which the most common one was congenital heart diseases (n=11, 34.4%). At presentation, intracardiac localization including inferior and superior vena cavae entrance was observed in almost one third of the patients (n=10, 31.2%). The thrombosis recurred at the same location in fifteen (47%) but at a different location in seventeen (53%). The median time interval between first and second episodes of thrombosis was 6.5 months (range; 1-180 months). Considering both acquired and congenital thrombophilic factors; three (9.3%), four (12.5%) and fourteen (43.8%) patients had 5 , 4 and 3 risk factors, respectively. More than half of the patients have higher plasma FVIII (> 150 IU/dl) & d-dimer (> 0.5mg/ml) levels. As a treatment option, thrombectomy was performed in three patients with organized, chronic intracardiac thrombus. Tissue plasminogen activator (t-PA) had been used more commonly at the recurrence (15.6% vs 28.1%) and the ratio of complete resolution was higher (40% vs 77.7%). The thrombus was partially resolved in eleven of the patients at initial episode and in ten at recurrence (34% vs 32%). Twenty-nine (87.5%) patients were on prophylaxis -by warfarin (n=16) and LMWH (n=12)- at the time of recurrence. Four patients (12.5%) died of their underlying disorders and six (18.7%) developed postthrombotic syndrome during follow up. **Conclusions.** The recurrent thrombosis should be predicted particularly in cases with congenital heart diseases or ongoing inflammatory processes such as malignancies and collagen vascular disorders. On the other hand; incomplete thrombus resolution and elevated plasma FVIII/d-dimer levels are also well known risk factors for rVTE. In the light of this knowledge; we suggest treating the patients with high recurrence risk aggresively.

0480

ROLE OF THROMBOSPONDIN IN THE INCREASED INCIDENCE OF VASOOCCLUSIVE CRISIS IN BLOOD GROUP 'O' SCD PATIENTS

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Background. Abnormal adhesive interactions between sickle erythrocytes and vascular endothelial cells (EC) and/or subendothelial matrix are known to play a dominant role in initiation of vasoocclusive events (VOC). Thrombospondin-1 (TSP) and von Willebrand factor (vWF) are the two most important mediators of the adhesive interactions between sickle erythrocytes and the blood vessel wall. Furthermore, TSP was also found more important than vWF to promote sickle cell adhesion to EC, but vWF was found to inhibit the effect of TSP. **Aims.** The aim of this study was to find if there is an increased incidence of VOC's in Blood Group "O"SCD patients, and if so, was it related to an upregulation of TSP along with a the relative reduction in the circulating vWF as compared to non-"O"blood group SCD patients. **Methods.** 89 consecutive SCD patients were enrolled in this study after an informed consent and institutional ethical clearance. Blood samples were obtained for vWF antigen, collagen binding activity, blood group typing, C-reactive protein, variant hemoglobin analysis (HPLC), Serum TSP levels, complete blood counts, liver function tests, LDH and renal function tests during VOC episodes and in steady state conditions. Disease severity was assessed by the annual frequency of inpatient admissions and disease complications. **Results.** Of the evaluable 71 patients, in the "O"blood group SCD subjects [n=36] the mean serum TSP$\pm$SD [Interquartile range] was significantly higher than the non-"O"blood group SCD subjects [n=35], namely, 38.8$\pm$28[22.4-38.4] v/s 25.5$\pm$12[17.5-27.6][p <0.05, Mann-Whitney test]. Furthermore, the serum TSP levels of 34.5$\pm$23 [22.6-36.6] in steady state conditions [n=72] were significantly reduced to 22.4$\pm$11[13.6-26.7] in patients presenting with acute vasoocclusive crisis [n=17] [p,<0.05, Mann-Whitney test]. **Summary and Conclusions.** It was observed that there was an inverse relation between TSP and vWF levels, in Blood group "O"SCD patients with an upregulation of the TSP levels leading to an increased propensity for vasoocclusion. However, during active VOC crisis, the TSP levels were significantly depressed.

0481

INPATIENT ENOXAPARIN FOLLOWED BY OUTPATIENT RIVAROXABAN USE IN ELECTIVE HIP AND KNEE ARTHROPLASTY: A SUCCESSFUL STRATEGY FOR EXTENDED THROMBOPROPHYLAXIS

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Background. Venous thromboembolism (VTE) is a serious complication after total hip or knee replacement surgery and studies have shown that this risk persists for many weeks. Extended thromboprophylaxis (TP) is now well recognised but parenteral administration of heparins pose a significant challenge. Treatment with vitamin k antagonists is also fraught with difficulties. Therefore

the recent availability and licensing of Rivaroxaban, a once-daily, fixed dose, oral anticoagulant for extended TP following elective orthopaedic surgery holds considerable promise. But, whilst clinical trials have demonstrated Rivaroxaban's safety and efficacy profile, experience from clinical practice is still limited. Additionally, concerns about higher bleeding rates and a lack of specific antidote to reverse its anticoagulant effect in the post operative period may hamper its widespread implementation amongst orthopaedic surgeons. **Aims.** We devised a thromboprophylaxis strategy using a combination of TP with Enoxaparin and Rivaroxaban at our orthopaedic department which would navigate these concerns. Enoxaparin has well established safety and efficacy profile and has been widely used for TP in orthopaedic and other settings. We administered Enoxaparin once daily in prophylactic doses during the inpatient phase, and on discharge from the hospital, Rivaroxaban was prescribed at a dose of 10mg once daily for 35 days after hip and 14 days after knee arthroplasty. **Methods.** Using our fully integrated electronic patient record system, we performed a 7 month retrospective audit for all elective THA and TKA performed at our centre with an aim to evaluate the incidence of VTE and bleedings rates in this cohort of patients. Each patient's records were evaluated for any evidence of VTE and or bleeding for up to 90 days post operatively. The primary end points were the incident of VTE and major and minor bleeding events. **Results.** Over a period of 7 months, 330 patients were evaluated. 167 were knee and 163 hip arthroplasty patients. Overall, mean age was 69.8 (age range 45-100), with 75.6% >65 years old, sex ratio was 184 female/145 male, and average duration of inpatient stay 5.18 days. After follow up of 90 days, only 2 VTE events were identified (0.60%). Both case were proximal DVT and occurred in patients after hip arthroplasty. Root cause analysis showed that the standard TP protocol was followed in both cases. Only one minor bleeding event was identified (0.30%) in the knee arthroplasty group with the development of a calf hematoma 25 days after discharge which was about 10 days after completing Rivaroxaban. None of the patient suffered from a major bleeding event, PE or mortality. **Summary and Conclusions.** Our data is a reflection of practical experience with this new oral anticoagulant and it indicates a low incidence of thrombotic and bleeding events which is no worse than primary endpoints reported in clinical trials of Rivaroxaban. Despite its limitations, our study suggests that the combination of Enoxaparin during in-patient phase followed by Rivaroxaban for extended prophylaxis can be an effective and safe strategy for TP after elective hip or knee arthroplasty and may allow better adherence to appropriate extended TP.

0482

PLASMINOGEN ACTIVATOR INHIBITOR-1 G4/G5 POLYMORPHISM AND RISK OF VENOUS THROMBOEMBOLISM: A DANISH FOLLOW-UP STUDY

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Background. Plasminogen activator inhibitor-1 (PAI-1) exerts a role in blood coagulation through inhibition of fibrinolysis and raised plasma levels of PAI-1 has been proposed as a risk factor for venous thromboembolism (VTE). Evidence suggests that the insertion/deletion polymorphism 4G/5G located in the promoter region of the PAI-1 gene locus affects PAI-1 plasma levels and the 4G/4G variant has been associated with a higher risk of VTE in some studies. Given the fact that obesity and smoking habits also modulate PAI-1 levels potential interaction between obesity, smoking and the PAI-1 polymorphism was explored. **Aims.** To investigate the association between the 4G/5G polymorphism and VTE as well as the interaction with obesity and smoking in a Danish follow-up study. **Methods.** From 1993 to 1997, 57.053 participants age 50 to 64 years and free from a prior diagnosis of cancer joined the Danish "Diet, Cancer and Health"cohort. The primary outcome of this study was a first-time verified VTE episode defined as deep venous thrombosis or pulmonary embolism, and participants with a diagnosis of VTE before enrolment were excluded. Detailed data on body mass index (BMI) and smoking habits as well as blood samples from each participant were obtained at baseline. G4/G5 genotypes were determined by allele specific real-time polymerase chain reactions (predesigned Taqman SNP genotyping assay from Applied Biosystems). A case-cohort design including all incident VTE cases and a randomly selected subcohort of 1841 participants from the whole cohort was applied. Cox proportional hazard model with age as time-axis were used for statistical analyses and adjustments were performed for smoking, BMI, sex, age and hormone therapy. **Results.** A total of 641 incident VTE cases were verified. Sufficient DNA was available for SNP genotyping in the majority of VTE cases and subcohort members. The 4G/4G variant was found in 25.8 % of participants in the subcohort, the 5G/5G variant was found in 24.2 % and the 4G/5G was found in 48.0%. The incidence rates (IR) of VTE and Hazard rate (HR) according to PAI-1 polymorphisms are shown in the Table. We found no association between VTE and PAI-1 genotype. There were no significant interactions between lifestyle factors and 4G/5G polymorphism and adjustments did not change the estimates. **Conclusions.** The present study could not confirm the previously reported association between the 4G/5G polymorphism and VTE and there was no apparent interaction between the 4G/5G polymorphism and common thrombogenic lifestyle factors.

Table 1. PAI-1 polymorphism and risk of VTE. Adjustment for BMI, smoking, sex, and use of hormones (women).

	PAI-1 polymorphisms		
	4G/4G	**4G/5G**	**5G/5G**
Number of cases (n)	157	291	147
Incidence rate	111.9 [93.6-134.4]	109.9 [96.3-125.6]	111.3 [92.5-134.5]
Crude HR	1.0 [reference]	0.94 [0.75-1.19]	0.96 [0.74-1.27]
Adjusted HR[#]	1.0 [reference]	0.95 [0.75-1.20]	0.97 [0.74-1.28]
Body Mass Index (BMI)	**PAI-1 polymorphisms**		
	4G/4G	**4G/5G**	**5G/5G**
BMI < 25 kg/m²	52	95	47
Number of cases (n)	79.3 [58.9-108.4]	83.5 [66.8-105.1]	75.2 [55.1-104.3]
IR	1.0 [reference]	1.01 [0.69-1.48]	0.90 [0.58-1.40]
Crude HR			
BMI ≥25 and <30 kg/m²	70	124	62
Number of cases (n)	121.6 [92.9-160.6]	106.6 [87.3-130.8]	121.5 [91.3-163.4]
IR	1.46 [0.97-2.22]	1.20 [0.83-1.74]	1.44 [0.94-2.21]
Crude HR			
BMI ≥ 30 kg/m²	34	70	37
Number of cases (n)	197.6 [129.9-304.3]	201.7 [150.2-272.3]	199.1 [132.8-301.7]
IR	2.20 [1.29-3.75]	2.43 [1.58-3.74]	2.23 [1.33-3.76]
Crude HR			
Smoking Status	**PAI-1 polymorphisms**		
	4G/4G	**4G/5G**	**5G/5G**
None Smoker	90	166	88
Number of cases (n)	104.8 [83.0-133.4]	99.4 [83.7-118.5]	106.7 [84.2-136.2]
IR	1.0 [reference]	0.93 [0.69-1.26]	0.98 [0.69-1.38]
Crude HR			
Moderate Smokers (1-24.9 g/d)	47	101	38
Number of cases (n)	112.4 [81.1-157.9]	125.4 [99.9-158.4]	89.2 [62.7-129.3]
IR	1.09 [0.72-1.65]	1.12 [0.80-1.57]	0.85 [0.55-1.32]
Crude HR			
Heavy Smokers (≥ 25 g/d)	19	22	20
Number of cases (n)	150.5 [88.6-263.6]	126.9 [78.6-210.9]	286.2 [158.2-522.1]
IR	1.52 [0.83-2.76]	1.20 [0.69-2.08]	2.90[1.50-5.61]
Crude HR			

0483

RELEVANCE OF CEREBRAL VEIN THROMBOSIS IN ADULTS TREATED FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Venous thromboembolism (VTE) frequently complicates the treatment of acute lymphoblastic leukemia (ALL). The reported incidence varies from 1.7% to 36.7% and seems higher in adult than pediatric patients. Occurrence is associated with disease, host and treatment modalities. A large proportion of VTE is located in the cerebral veins (CVT), however the reason for this preference is unknown. **Aims.** To determine etiological and clinical associations of CVT, we analyzed incidence, relationships with treatment elements, and clinical characteristics and outcome in a large cohort of adults treated for ALL. **Methods.** A post-hoc analysis was performed in patients treated for newly diagnosed ALL in a multicenter study (HOVON-37 ALL). 240 patients aged 16-59 years were included between April 1999 and November 2005. All symptomatic objectified VTE was prospectively recorded. For patients with CVT, clinical parameters were extracted from hospital records. **Results.** Nine patients experienced CVT (3.8%; 95% confidence interval (CI) 1.4-6.2). One of nine patients had a central nervous system localisation of ALL, while four of nine had a poor ALL prognostic risk classification, based on presence of cytogenetic abnormalities (t(9;22), t(4;11), or t(1;19)), pro-B-cell immunophenotype, and high WBC (i.e. > 30 x 10^9/L in case of B-cell ALL; > 100 x 10^9/L in case of T-cell ALL). All CVT occurred during cycle 1 of remission induction treatment and CVT was located in the superior sagittal sinus in eight of nine patients. Eight

patients presented with seizures and six had focal neurological defects upon presentation. Seven patients had parenchymal brain lesions and two had significant mass effect due to hemorrhagic infarction. Median time between inclusion and CVT was 21 days (range 13-33), 0 days (range 0-2) between last L-asparaginase administration and 6 days (range 1-13) between last intrathecal methotrexate administration and CVT. L-asparaginase was discontinued if CVT occurred during L-asparaginase therapy, and withheld in one patient due to CVT. All patients but one were treated with anticoagulation after CVT. Two patients also received endovascular thrombolysis preceding anticoagulation. However, both died in the acute phase due to transtentorial herniation as a result of increase in hemorrhagic infarction. Patients with CVT were less likely to obtain complete remission than patients without CVT after cycle 1 (odds ratio (OR) 0.17; 95% CI 0.03-0.82; $p = 0.01$) and on protocol overall (OR 0.13; 95% CI 0.03-0.72; $p < 0.01$). **Conclusions.** CVT is a relative frequent complication during ALL treatment in adults. The clinical presentation of CVT in ALL seems more severe than in other CVT events, with a high mortality rate, and common seizures, hemorrhagic infarctions and localisation in the superior sagittal sinus. Our study suggests etiological relationships with L-asparaginase and intrathecal methotrexate therapy in the first remission induction cycle. Future studies are necessary to confirm these results and to clarify underlying mechanisms.

0484

DEALING WITH THE GREY AREA: BORDERLINE RESULTS IN PROTEIN C, PROTEIN S AND ANTITHROMBIN TESTING

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Background. Protein C (PC), Protein S (PS) and Antithrombin (AT) deficiencies carry an increased risk of thrombosis, and levels are tested during thrombophilia workup; however, there are marked inter and intra-individual biological variations in measurements, even in the absence of disease.Normality is a continuum. However, “normal”values are determined statistically for lab tests, with artificial boundaries separating “normal”from “altered”results. In tests with high intra-individual variation, the patient can fall on either side of the boundary between “normal”and “not-normal”on sequential determinations.Assuming adequate quality-control, results with a severe deviation from normal are of easy interpretation; however, altered results can fall into a grey area between “normal”and “low”that requires further investigation. We aim to characterize the difficulties these results raise for the clinician, by analyzing the occurrence of falsely positive values in borderline-low results. **Methods.** We reviewed all requests for free PS, PC and AT received in our lab between 01-Jan-1999 and 26-Feb-2012, defining a Normal interval of 60-130% for PS, 70-140% for PC and 80-120% of AT. “Borderline”was within a 10-percentual-unit range of the lower limit of normal. **Results.** Reviewing 5938 results for PS, 5900 for PC and 5568 for AT, we found that 82.9%, 80.7% and 82.2% were Normal, respectively, while 2.8%, 8.3% and 6.3% were above Normal. Values below 20% accounted for 0.51%, 0.44% and 0.07%, while 5.6% of samples were Borderline (6.2% PS, 3.5% PC and 7.1% AT).A total of 682 patients were repeatedly tested for PS and PC, and 616 for AT; the average intra-individual variation-coefficients were 11.7%, 9.7% and 6.3%.Considering Borderline results for PC, 56 patients had repeated testing: 46.4% were previously Normal, 7.1% were previously low and 44.6% had no prior assays; 32.1% had no follow-up assay, 42.9% were Normal, 14.3% remained Borderline and 10.7% were low. For PS, of 95 repeatedly tested patients, 32.7% were previously Normal and 17.9% previously low; at follow-up 48.4% were Normal, 11.6% remained Borderline and 11.6% were low. For AT, 36.1% of 97 repeat patients were previously Normal, 8.2% previously low; 52.6% Normal at follow-up, 11.3% Borderline and 4.1% low. **Discussion.** We observed that 86-89% of results (85.7% PS, 88.9% PC and 88.4% AT) obtained were either Normal or above Normal, carrying no thrombotic risk and being, therefore, of easy interpretation, while under 0.5% of results were also of easy interpretation, falling below 20%.However, 4-7% of results (6.2% PS, 3.5% PC and 7.1% AT) fell into a grey area below the boundary of normal, requiring further investigation. The uncertainty of results within this borderline-low area was demonstrated by the fact that 33-47% of patients were previously normal and 7-18% were previously low, and that 43-53% had subsequent normal assays, while 15-25% were low-to-borderline-low, findings compatible with the high intra-individual variation-coefficients obtained (6%-12%).Our results reinforce the notions that when interpreting isolated thrombophilia test results, an adequate patient history is fundamental, and that confirmatory assays should be obtained before establishing a diagnosis, moreover when results fall into borderline values.

0485

TISSUE FACTOR EXPRESSION PREDICTS OUTCOME IN CHILDREN WITH NEUROBLASTOMA

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Background. It has recently been proposed that oncogenic changes may impact the level of tissue factor (TF) and thereby affect cancer coagulopathy, angiogenesis and other vascular effects associated with cancer. For this notion, there is already a growing body of experimental and early clinical evidence. For instance, TF upregulation parallels the expression of several mutant oncogenes and this was documented in cancer cells of colorectal, mammary gland, cutaneous, astrocytic and hematologic origin. **Aims.** To the best of our knowledge we report for the first time the detection of tissue factor expression by immunohistochemistry in neuroblastoma in pediatrics and its correlation with tumor pathology, stage and clinical outcome. **Methods.** Twenty children registered in Pediatric Oncology Unit of Zagazig University Hospital with biopsy proven neuroblastoma were studied retrospectively. The patients were enrolled and treated on COG risk- based protocol during the period from January 2006 to February 2011. We collected full clinical, laboratory, imaging and tumor data. Data regarding response to treatment and outcome were also collected. TF expression level was detected on formalin-fixed, paraffin -embedded tissue sections. **Results.** Increased tissue factor expression by immunohistochemistry was detected in 75% of our patients. 75% of patients with stage IV neuroblastoma were expressing TF and 67% of patients with stage III had TF expression by immunohistochemistry. There was no significant relationship between TF expression and each of age, gender, clinical and laboratory findings. Although higher percentage of unfavorable Shimada pathology was noted in patients with positive tissue factor expression than those with negative TF expression (60% versus 20%), still the difference was insignificant. None of patients with negative TF expression had MYCN amplified while 62% of those with positive TF expression and known MYCN status had MYCN amplification.TF expression was significantly related to patient outcome where all patients with negative tissue factor expression cured while 81.8% of those with positive tissue factor expression and known outcome, died. There was no significant correlation between intensity of staining of TF and pathology, stage nor age. **Summary and Conclusions.** Tissue factor is a promising research subject as a prognostic factor for neuroblastoma tumor. More studies are needed to clarify the mechanisms by which tissue factor affects cancer progression and outcome, and its potential role as a therapeutic target.

0486

COAGULATION PROFILE OF ET PATIENTS PRESENTING THROMBOTIC COMPLICATIONS

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Background. ET is a relatively indolent disease but a reduction of survival has been observed. The relative survival ratio at 10 years is 0.68 (0.64-0.71) for ET patients. The main risk factors for reduced survival are advanced age and the history of thrombotic complications. Therefore, it is essential to search for main risk factors of thrombosis. **Aims.** The study was designed to identify a characteristic coagulation profile of ET patients presenting thrombotic complications. **Methods.** We have examined 106 patients with ET, 80 females and 26 males, mean age 54 (23-82) yrs. The control group (CG) consisted of 20 healthy persons: 6 males and 14 females, mean age 41 (31-54) yrs. Thrombotic complications (TC) occurred in 21 ET patients, bleeding episodes (BE) were noticed in 22 ET patients. Thrombosis is caused by a variety of reasons. We put them into several main groups to find which the most common ones are:1. Gene mutations: JAK-2 V617F; factor V Leiden, G20210A prothrombin, MTHFR C667T and PAI-1 polimorphism.2. Coexistence of at least one of cardiovascular risk factors: diabetes, hypertension, smoking.3. Proteins level below normal range: C, free S, AT and F XII.4. F I, F VIII, vWF and homocysteine levels above normal range as well as abnormal lipid profile.5. Additional factors: age >60, BMI >30, WBC (at diag.)>8.4G/l, Ht >45%.Additionally,

besides evaluating PF4 concentration in patient's serum we assessed its concentration in platelet lysates. **Results.** The median age of TC patients was over 60 and higher than the median age of BE patients: 61yrs; 48-67 vs 48.5yrs; 36-60; p<0.05 (all results are expressed by median and P25-75%). They also present more thrombotic risk factors (TC:7; 5-9 vs BE: 4; 3-5; p<0.001). vWF activity and antigen concentration as well as factor VIII level were higher in TC than in BE patients (87.4%; 67-134 vs 66.5% 48.9-87.4 and 127% 99-153.5 vs 94.6%; 72-128.97, and 85%; 81.3-122.5 vs 79.5%; 61.05-92.8; p<0.05). As much as 14 (32.56%) patients with TC were JAK-2 positive as compared to only 5 (11.63%) BE patients. Moreover, JAK2 positive patients with TC had lower free protein S levels as compared to JAK2 negative patients. The median level of fibrinogen in TC patients was over 350mg/dL and higher, as compared to ET patients without any complications (353mg/dL; 290-409 vs 281mg/dl; 240-340 mg% p<0.05).The median PF4 conc. in platelet lysates was 12.78 IU/l; 10.07-17.35 for the whole ET group, 15.25 IU/L; 11.37-15.567 for CG , 15.6 IU/L; 10.41-17.68 for TC patients , 11.34 IU/L; 9.93-13.56 for BE patients and 14.17 IU/L; 9.99-17.13 for patients without any vascular complications. **Conclusions.** *1.*Patients with TC are older, more often JAK-2 positive and present more thrombotic risk factors as well as higher FVIII, and vWF levels compared to patients with BE.2. Although BE patients have lower PF4/1mln platelets ratio than TC patients (our previous results), the concentration of PF4 in platelets lysates was the same in all ET patients groups irrespective of presence or type of vascular complications.

0488

PROTHROMBIN G20210A AND ORAL CONTRACEPTIVES AS RISK FACTORS FOR CEREBRAL VENOUS THROMBOSIS

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Background. Prothrombin variant G20210A (PT G20210A) and the use of oral contraceptives (OC) are major risk factors for cerebral venous thrombosis (CVT). A synergism between these factors has also been reported. However, the role of factor V Leiden (FV Leiden) and PT G20210A as independent risk factors for CVT according to gender and the intensity of the synergism with OC remain controversial. **Aims.** This study evaluated the association between FV Leiden and PT G20210A with CVT in each gender and the interaction with oral contraceptives. The results were also compared with those observed in patients with venous thromboembolism (VTE).**Methods.** 91 patients (73 women and 18 men) diagnosed with CVT were studied. A control group of 284 individuals with no history of thrombosis (matched by age and sex) and a group of 343 patients (218 women and 125 men) with VTE were also studied. Cases and controls were tested for the presence of FV Leiden and PT G20210A. **Results.** The frequencies of FV Leiden, PT G20210A by gender and the interaction with oral contraceptive use are showed in Table 1. **Conclusions.** Our results confirm that PT G20210A is an independent risk factor for CVT, with a higher risk than in patients with VTE. The risk was similar in men and women, and was higher than that of OC use alone. The association of PT G20210A with OC use had a strong synergistic effect. In contrast with the observed for VTE FV Leiden was not a risk factor for CVT.

Table 1. Frequencies of prothrombin G20210A, factor V Leiden and oral contraceptive use in patients and controls.

Variables	CVT patients	Controls	OR 95% CI	VTE patients	Controls	OR 95% CI
			MEN			
N	18	110		125	110	
FV	1	2	3,2(0,3-37)	21	2	10 (2,5-47)
PT	2	1	14 (1-162)	12	1	11 (1,5-90)
			WOMEN			
N	75	176		218	176	
FV	3	3	2,4 (0,5-12)	33	3	10 (3-34)
PT	12	3	12 (3-43)	19	3	5,5 (1,6-19)
OC	49	70	3 (1,5-4,8)	143	70	2,8 (2-4)
PT + OC	10	1	43 (5–346)	10	1	14 (2-115)

CVT - cerebral venous thrombosis; VTE - venous thromboembolism; FV - factor V Leiden; PT - prothrombin G20210A; OC - oral contraceptives; OR – odds ratio; CI - confidence interval.

0489

HEMOSTATIC AND METABOLIC PARAMETERS IN PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM: IS THERE A RELATIONSHIP WITH THYROID STIMULATING HORMONE?

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Background. Previous data regarding abnormalities of blood coagulation during hypothyroidism and hyperthyroidism have been published. Nevertheless, a controversy exists concerning the presence of a hypercoagulable status in these disease states. Subclinical hypothyroidism (SH) is often encountered in the general population. Patients may be asymptomatic or may present non-specific symptoms. It has been suggested that SH could represent a risk factor for cardiovascular disease, especially coronary heart disease. Hemostatic and metabolic parameters as well as their relationship with thyroid stimulating hormone (TSH) have not been studied in depth in subclinical hypothyroidism. **Aims.** The aim of the present study was to evaluate hemostatic and metabolic perturbations in patients with SH in comparison to euthyroidic healthy subjects, and to investigate whether serum TSH and thyroid hormones may contribute to the variation of hemostatic parameters. **Methods.** In a cross-sectional study that we conducted between 2009 and 2011, we have evaluated forty three patients with subclinical hypothyroidism prior to any therapeutic intervention with levothyroxine (33 women and 10 men) with a mean age: 34.8 ± 8.3 years (range: 18-50 years) and an equal number of euthyroidic healthy subjects (33 women and 10 men) with a mean age: 33.9 ± 8 years (range: 20-48 years). Healthy subjects were matched on gender, age (±5 years), body mass index (± 1.5 kg/m^2) and year/month of diagnosis (±1 month). None of the subjects (patients and controls) presented any infectious and neoplastic conditions, diabetes mellitus, hypertension and dyslipidemia. Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, vWF, plasminogen, antithrombin III (ATIII), protein C and S were evaluated using immunonephelometry (Dade-Behring). Lipid variables such as triglycerides, total cholesterol (C), LDL-C and HDL-C were also assessed. To evaluate thrombopoiesis, we have determined platelet indices (mean platelet volume, MPV; platelet distribution width, PDW; platelet count) using the Sysmex 9000 analyzer. TSH, T3, free-T3, T4 and free-T4 were determined using an electro-chemiluminescence immunoassay intended for use on Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, USA). Statistical analysis of data was performed with IBM-SPSS® statistical package version 20. **Results.** Cases with SH presented significantly higher MPV (mean ± SD: 12.31 fL ± 0.45) and PDW (mean ± SD: 15.3 % ± 1.1) than controls (mean MPV ± SD: 10.7 fL ± 0.82, p<0.001 and mean PDW ± SD: 13.4 % ± 1.42, p<0.001). Also, cases exhibited a significantly decrease in PT (p=0.043) and HDL-C (p=0.045) and significant increases in fibrinogen (p=0.05), triglycerides (p=0.023) and total cholesterol levels (p=0.045). Moreover, serum TSH levels were correlated with MPV (Spearman r=0.63, p<0.001) and PDW (Spearman r=0.42; p=0.024) in patients with SH but not euthyroidic healthy controls. No other significant correlations were found between thyroid hormones and hemostatic variables (p>0.05). **Conclusions.** This study suggest that subjects with SH tend to present minor coagulation abnormalities. Elevated platelet activation in conjunction with minor coagulation and lipid perturbations could contribute to an increased risk of cardiovascular complications observed in SH. Finally, the findings of this study suggest that platelet morphologic changes observed in SH may be attributed to hormonal parameters.

0490

SYSTEMIC MULTI-ORGAN COMPLICATIONS IN ATYPICAL HEMOLYTIC UREMIC SYNDROME (AHUS): RETROSPECTIVE STUDY IN A MEDICAL PRACTICE SETTING

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Background. Atypical hemolytic uremic syndrome (aHUS) is a genetic and life-threatening disease, in which permanently uncontrolled complement activation leads to platelet activation, endothelial damage, and multiple thrombotic occlusions in small vessels throughout the body (a process called systemic thrombotic microangiopathy, or TMA). Historically, the prognosis for aHUS patients is very poor and despite plasma exchange/infusion (PE/PI), 33%-40% of patients progress to end-stage renal disease or death with the first clinical aHUS manifestation and 65% of patients require dialysis, have permanent kidney damage, or die within 1 year of diagnosis. Though kidney impairment and end-stage renal disease are recognized as severe complications of aHUS, pre-

vious reports of cardiovascular (43%) and neurologic (48%) complications in the medical literature highlight the significant extra-renal morbidity that results from ongoing TMA in other vital organs. **Aims.** To identify and describe evidence of systemic multi-organ aHUS complications in a cohort of patients in the medical-practice setting. **Methods.** Medical record data were retrospectively collected for 30 pediatric, adolescent, and adult aHUS patients who received eculizumab treatment between 2007 and 2009 outside of a controlled clinical trial setting. Data corresponding to the time period prior to first eculizumab dose are described. **Results.** aHUS disease characteristics for patients at study baseline (prior to administration of first dose of eculizumab) are shown in Table 1. Medical records of all 30 aHUS patients (100%) showed evidence of kidney impairment during the time period prior to eculizumab treatment. Extra-renal, systemic organ complications were reported in 19 patients (63%; Table 2). Eleven patients (37%) experienced extra-renal thrombi, including deep vein thrombosis (n=5 patients [distal lower extremity, upper arm, shoulder, right iliac vein, nonspecified location]), myocardial infarction and pulmonary embolism (n=1), transient ischemic attacks (n=1), stroke (n=1), splenic vein occlusion (n=1), central retinal vein occlusion (n=1), and multiple thromboses (n=1). Four of these 11 patients experienced thrombotic events with no history of dialysis. In addition to extra-renal thromboses, cardiac morbidities included cardiac arrest (n=2) and cardiomyopathy (n=2). Gastrointestinal signs/symptoms were associated with aHUS presentation in 11 patients (37%) and included diarrhea (n=8), vomiting (n=5), and pancreatitis (n=2) together with the splenic vein occlusion (n=1) described above. Neurologic complications were found in this cohort, including seizures (n=2, including 1 patient with acute disseminated encephalomyelitis), headache (n=2), and facial paralysis (n=1), as well as the extra-renal thromboses of stroke and transient ischemic attacks (n=1 each) above. **Conclusions.** aHUS is a devastating and progressive disease in which TMA can be associated with sudden and potentially fatal systemic aHUS morbidities. These data are consistent with previous reports of multi-organ aHUS complications due to systemic TMAs, and suggest that evidence of TMA or progressive systemic organ involvement (including cardiac, gastrointestinal, and neurologic complications, as well as peripheral thrombi) should prompt high suspicion of aHUS as a clinical diagnosis, even in the absence of kidney failure.Craig Langman: c-langman@northwestern.edu

Table 1. Baseline[a] aHUS Disease Characteristics

Characteristic	All Patients (N=30)
Duration from aHUS diagnosis to 1st eculizumab dose, months, median (range)	11 (<1–176)
Platelet count <150×10⁹/L, n (%)	13 (43)
Serum LDH >ULN, n (%)	20 (67)
eGFR mL/min/1.73 m², n (%)	
≥90	4 (13)
60 to <90	2 (7)
30 to <60	11 (37)
15 to <30	5 (17)
<15	8 (27)
Received dialysis in 4 weeks prior to baseline, n (%)	11 (37)
Previous kidney transplantation, n (%)	11 (37)
Identified genetic complement mutation, n (%)	14 (47)

[a]Prior to initiation of eculizumab treatment. aHUS=atypical hemolytic uremic syndrome; eGFR=estimated glomerular filtration rate; LDH=lactate dehydrogenase; ULN=upper limit of normal.

Table 2. Incidence of aHUS Complications by System

System	Signs/Symptoms	No. (%) Patients
Renal	Kidney impairment	30 (100)
Cardiovascular	Thrombi (various locations), cardiac arrest, cardiomyopathy	11 (37)
Gastrointestinal	Diarrhea, vomiting, pancreatitis, splenic vein occlusion	11 (37)
Neurologic	Seizure, acute disseminated encephalomyelitis, stroke, transient ischemic attacks, facial paralysis, headache	5 (17)
aHUS complications in >1 system		**19 (63)**

Platelets 1

0491

PLATELET-ASSOCIATED ANTI-GPIIB/IIIA AUTOANTIBODIES IN PRIMARY IMMUNE THROMBOCYTOPENIA RECOGNIZE HIGHLY RESTRICTED REGIONS IN THE BETA-PROPELLER DOMAIN OF GPIIB WITH CLONALITY

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Background. Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by thrombocytopenia that results from immune-mediated platelet destruction and reduced platelet production. Platelet-associated (PA) anti-GPIIb/IIIa autoantibodies (Abs) play a key role in its pathogenesis. We and others have reported that epitopes of PA anti-GPIIb/IIIa Abs are mainly localized on GPIIb. However, the details of the epitopes have not been elucidated. **Aims.** To characterize epitopes of PA anti-GPIIb/IIIa Abs. [Methods] Platelet eluates from 76 patients with primary ITP (21 males, 55 females) were obtained by diethylether elution method. (We obtained written, informed consent for blood sampling from all patients.) We explored PA anti-GPIIb/IIIa Abs reactivity to human-mouse chimeric GPIIb/IIIa, since we noticed that the PA anti-GPIIb/IIIa Abs had markedly impaired reactivity to mouse GPIIb/IIIa. Human-mouse chimeric GPIIb/IIIa was transiently expressed in 293T cells and IgG binding was detected by flow cytometry. We also examined the light chain usage of the PA anti-GPIIb/IIIa Abs by flow cytometry. **Results.** PA anti-GPIIb/IIIa Abs were detected in 26 patients (34%) out of 76 patients, and we further analyzed 15 patients. We first found that the epitopes of PA anti-GPIIb/IIIa Abs were mainly localized in the N-terminal half of the β-propeller domain (L1-W235) of GPIIb. Next, using chimeric GPIIb in which surface residues of the N-terminal half of the β-propeller domain were serially exchanged with the corresponding mouse sequences, we could identify three major epitopes of PA anti-GPIIb/IIIa Abs; 1) Group A: The PA anti-GPIIb/IIIa Abs in 2 patients recognized W1:1-2 and W2:3-4 loops. W1:1-2 loop was very close to the W2:3-4 loop on the lower face of the β-propeller domain. Interestingly, the PA Abs reactivity was abolished by the following single amino acid substitution in GPIIb from human to mouse; S29K and R32S in W1:1-2 and E136Q and R139G in W2:3-4. 2) Group B: The PA anti-GPIIb/IIIa Abs in 5 patients recognized W1:2-3 loop. The PA Ab of one patient exclusively recognized the W1:2-3 loop and the PA Ab reactivity was markedly impaired by G44N and P45A substitutions in the loop. The PA Abs of other two patients mainly recognized W1:2-3, W2:3-4, and W3:3-4 loops, and the PA Abs from the remaining two patients recognized W1:2-3 and W2:3-4 loops. The PA Abs reactivity of these four patients was markedly impaired by R139G substitution in W2:3-4 like Group A patients. 3) Group C: The PA anti-GPIIb/IIIa Abs in 4 patients recognized W3:4-1 loop, which is near the ligand binding site, and furthermore 3 patients of them also recognized W4:4-1 loop which was immediately adjacent to the W3:4-1 loop. The PA anti-GPIIb-IIIa Abs in the remaining 4 patients also mainly recognized the N-terminal half in the β-propeller domain of GPIIb, but they could not be classified in the three groups described. Moreover we observed that many of the PA anti-GPIIb/IIIa Abs exhibited restricted usage of κ/λ light chain. **Summary**. We have demonstrated that many PA anti-GPIIb/IIIa Abs recognized highly restricted regions in the N-terminal half of the β-propeller domain of GPIIb with clonality.

0492

SPECIFIC BINDING OF PARAPROTEIN TO PLATELET RECEPTORS AS A CAUSE OF PLATELET DISFUNCTION IN MONOCLONAL GAMMOPATHIES

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Background. About 10% of patients with monoclonal gammopathies (MG) have hemostatic disorders at presentation, while the results of platelet function tests are abnormal in 30-55% of cases. The arising mechanism is believed to be connected with paraprotein. **Aims.** Our objectives were to examine the role of paraprotein in the appearance of abnormalities in platelet aggregation, and

to determine specific mechanisms whereby paraprotein inhibits normal platelet function in patients with MG. **Methods.** The study included 48 untreated patients with MG. Platelet aggregation was initially, induced with collagen (COL) and ristocetine (RIS). Paraprotein was isolated from the serum of ten patients with poor platelet aggregation. Platelet aggregation was measured before and after addition of the isolated paraprotein to platelet rich plasma (PRP) from ten healthy donors, *in vitro*. Expression of platelet vWF receptor-glycoprotein (GP) Ib and platelet collagen receptor GPVI was determined by flow cytometry in PRP of healthy donors before and after addition of isolated paraprotein using the monoclonal antibodies, CD42b (for GPIb), and CD36 (for GPVI). To evaluate surface changes on platelets, blood samples were mixed with the following mouse monoclonal antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-CD36 and phycoerythrein (PE)-conjugated anti-CD42b. Peridinin Chlorophyll Protein Complex (PerCP)-conjugated anti-CD61 (specific for GPIIIa) was used to identify the platelet population. Appropriate isotype-matched controls were run in parallel to all monoclonal antibodies. The proportion of positive (%Pos) cells and the mean fluorescence intensity (MFI) were obtained for each sample as an index of receptor expression. The results were then expressed as total fluorescence intensity (TFI), where TFI = (%Pos/100) x MFI. As previously described, TFI takes into account both the %Pos cells and MFI.The same tests were repeated before and after addition of human immunoglobulins for intravenous use to PRP of healthy donors. **Results.** Platelet aggregation with COL (p = 0.008) and RIS (p = 0.001) in PRP of healthy donors was significantly decreased after addition of paraprotein. Flowcytometry showed that expression of CD42b and CD36 positive cells was significantly lower after addition of isolated paraproteins to PRP from healthy donors (p<0.001), while expression of CD61 positive cells was not changed. Also, MFI and TFI for both antibodies (CD42b and CD36) declined significantly after addition of paraprotein to PRP of healthy donors. In comparison, when human immunoglobulin was added to PRP of healthy donors, neither platelet aggregation nor expression of CD42b and CD36 positive cells nor MFI and TFI were altered. **Conclusions.** These investigations demonstrated that paraprotein causes platelet disfunction in patients with MG due to specific binding to the platelet vWF receptor GPIb and platelet collagen receptor GPVI. Specific binding of paraprotein to these platelet receptors was confirmed by the markedly decreased expression of CD42b and CD36 positive cells without changes in expression of CD61 positivity. By this mechanism paraprotein disables the normal function of platelets.

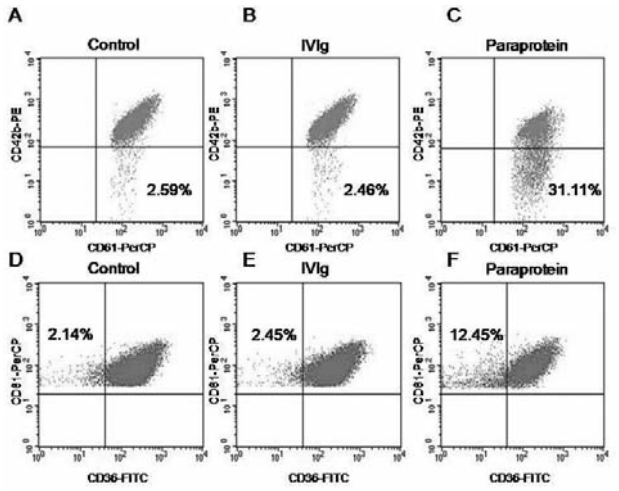

Figure 1. Decreased expression of CD42b and CD36 positive cells after addition of paraprotein in platelet rich plasma of healthy donors by flowcytometry.

0493

ANALYSIS OF BASELINE BONE MARROW CHARACTERISTICS IN A LONGITUDINAL 2-YEAR BONE MARROW STUDY OF ELTROMBOPAG IN PREVIOUSLY TREATED PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Background. Bone marrow biopsies are not routinely performed in the management of chronic immune thrombocytopenia (cITP). Although some studies have retrospectively reviewed biopsy specimens, prospectively collected data in cITP are limited. Reticulin grades 0, 1, and 2 (Bauermeister scale) have been reported in healthy volunteers in 7%, 73%, and 20%, respectively [Sundström 2002]. A Danish study of 187 cITP patients never treated with thrombopoietin receptor agonists (TPO-RAs) showed that 60% had MF-0, 38% MF-1, and ~2% MF-2 or MF-3 (European Consensus scale) [Ettrup 2010]. TPO-RAs have been associated with varying degrees of increases in reticulin fibers [Brynes 2011, Ghanima 2011], but the lack of prospectively collected and consistently stained and analyzed pre-treatment bone marrow specimens has limited the robustness of the conclusion. This study was designed to prospectively investigate potential effects of eltrombopag on the bone marrow. **Aims.** To assess the degree of baseline bone marrow reticulin and collagen in adults with cITP. **Methods.** Adults with cITP who failed first-line therapy were enrolled in this open-label, 2-year study (NCT01098487). Bone marrow biopsies were collected at baseline and after 1 and 2 years of eltrombopag treatment. Specimens were centrally processed and underwent a central pathology review. They were assessed for cellularity; megakaryocyte, erythroid, and myeloid quantity and appearance; trabecular bone quality; reticulin grade; and collagen deposition (European Consensus scale) [Thiele 2005]. Baseline findings of the bone marrow biopsies are reported. **Results.** One hundred fifty-five patients were analyzed. Median age was 41 years (18-80); 100 (65%) were female. Median time since cITP diagnosis was 3.9 years (0.5-45.7). 143 (92%) patients were TPO-RA-naive and 12 (8%) had received prior TPO-RA treatment (eltrombopag or romiplostim), the last dose at least 6 months prior to enrollment. 34 (22%) patients were splenectomized. Median platelet count prior to bone marrow biopsy was 21,000/μL (0-222,000/μL).Cellularity was normal (40-60%) in 130 (85%) patients (Table 1). 139 (91%) patients demonstrated megakaryocytic hyperplasia. Megakaryocyte morphology was normal in 144 (94%) patients. Erythroid hyperplasia was observed in 27 (18%) patients and granulocytic maturation was hyperplastic in 7 (5%) patients.Trabecular bone thinning was found in 60 (43%) patients, the majority with prior exposure to corticosteroids. Reticulin fibers were graded as MF-0 in 139 (90%) and MF-1 in 16 (10%) patients. Reticulin fibers were graded as MF-0 for all 12 patients who had received prior TPO-RA treatment. No patients demonstrated collagen deposition. **Summary and Conclusions.** Analysis of this prospectively collected database indicates that approximately 10% of cITP patients may have MF-1 reticulin in their bone marrow. It has been hypothesized that TPO-RAs may increase reticulin and collagen deposition in the bone marrow. Data from the EXTEND study presented in 2011 [Brynes 2011] do not indicate a clinically relevant increase in reticulin; however, the analysis was limited by lack of baseline bone marrow data. Prospective data from the current study will allow a better understanding of the potential effect of eltrombopag on the bone marrow.

Table 1. Baseline bone marrow biopsy analyses.

Analyses			
Lineage-specific	**Megakaryocytic**	**Myeloid**	**Erythroid**
Quantity, n (%)			
n	153	154	153
Normal	12 (8)	145 (94)	125 (82)
Increased	139 (91)	7 (5)	27 (18)
Decreased	2 (1)	2 (1)	1 (<1)
Quality, n (%)			
n	153	154	154
Normal maturation	144 (94)	153 (99)	153 (99)
Left shift	9 (6)	1 (1)	1 (1)
Cellularity, n (%)			
n		153	
Normal		130 (85)	
Increased		19 (12)	
Decreased		4 (3)	
Trabecular bone quality, n (%)			
n		139	
Normal		79 (57)	
Thinning		60 (43)	
Reticulin grade*, n (%)			
n		155	
MF-0		139 (90)	
MF-1		16 (10)	
MF-2		0	
MF-3		0	

*European Consensus scale.

0494

SAFETY AND EFFICACY OF FIXED DOSE RITUXIMAB IN PATIENTS WITH REFRACTORY, RELAPSING OR CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA, R-ITP1000 STUDY-FINAL ANALYSIS

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Background. Idiopathic thrombocytopenic purpura (ITP) is an auto-immune disorder characterized by low platelet count and mucocutaneous bleeding. Post-acute presentation, 25-30% adult patients develop chronic ITP, up to 30% of whom become refractory to corticosteroids and require additional therapy. B-cells play an important pathophysiological role in auto-immune disease. Rituximab depletes CD20+ B-cells and an exploratory dosing regimen based on lymphoma therapy (375 mg/m^2 weekly intravenous x4) has shown promising efficacy (~38% overall response rate-ORR) in adults with chronic and relapsing ITP. We explored the effectiveness of an abbreviated fixed dose rituximab schedule that has been approved in rheumatoid arthritis. Rituximab is not currently approved for the treatment of ITP. **Aims.** The primary objective was to determine the ORR at week 8 in adults (≥18 years), with chronic or relapsing ITP (platelet count >10x10^9/L and ≤50x10^9/L) according to the ASH guidelines, who received rituximab 1000mg on days 1 and 15. Secondary objectives included time to, duration of and incidence of therapeutic response (defined below). **Methods.** After obtaining informed consent, patients received scheduled rituximab and were followed-up for at least 12 weeks. Evaluations were at mandatory follow-up visits on weeks 8, 12, 26, 39 and 52. ORR was defined as the proportion of patients achieving complete response (CR, platelet count >150x10^9/L) or partial response (PR, >50x10^9/L) at week 8 with 2 consecutive measurements, confirmed at least 2 weeks apart. Minor response (MR) was defined as platelet count >30x10^9/L. Simon's 2-stage design was used to determine if the ORR was more likely to be ≤38% or ≥50%. At least 50 out of 108 responders (46%) were required to conclude, with 95% confidence and 80% power, that the ORR was more likely to be ≥50%. Duration of response was determined for patients with a response (MR/PR/CR) at week 8. Time to response was defined as time from first rituximab infusion to time of onset of MR/PR/CR, at any time throughout the study. Therapeutic response was defined as achieving a week 8 response, with at least minor response sustained through to weeks 26 and 52, and reduction in ITP medication.

Table 1. Incidence of response at week 8, duration of response, time to response and time to initiation of new ITP therapy.

Incidence of Response at Week 8 (N = 108)		Patients with relapse n (%)	Patients without relapse n (%)	Median duration of response (days)	95% CI
CR	N = 10	1 (10)	9 (90)	ND	(ND, ND)
CR+PR	N = 47	25 (53)	22 (47)	247	(219, ND)
CR+PR+MR	N = 66	33 (50)	33 (50)	256	(168, ND)
Time to Response at any visit		**Patients with response n (%)**	**Patients without response n (%)**	**Median time to response (days)**	**95% CI**
CR		30 (28)	78 (72)	ND	(305, ND)
CR+PR		61 (57)	47 (44)	53	(36, 152)
CR+PR+MR		77 (71)	31 (29)	22	(8, 30)
Time to New ITP Therapy*		**Patients with new therapy n (%)**	**Patients without new therapy n (%)**	**Median time to new therapy (days)**	**95% CI**
Any New ITP Therapy		54 (50)	54 (50)	314	(215, ND)

ND= not determined due to insufficient events. *Including patients who did not respond at week 8.

Results. Of 124 recruited patients, 2 did not receive study medication and 14 did not have platelet count ≤50x10^9/L within 7 days of first rituximab dose and were excluded from analysis. At week 8, the ORR was 44% (47/108 patients). Median duration of response, time to response and time to initiation of new ITP therapy results are presented in Table 1. Therapeutic response at weeks 26 and 52 was achieved in 47(44%) and 38(35%) patients, respectively. Treatment was well tolerated with no new safety signals reported. **Conclusions.** The observed response rate and durability of response in this study compare favorably with rituximab given weekly for 4 weeks, and offers relapsing/chronic ITP patients an alternative to ongoing thrombopoietin receptor agonists. Further investigations are warranted to determine whether the same response can be achieved with single/lower dosing rituximab or whether longer/more intense dosing, as well as maintenance dosing, might improve ORR and durability of responses.

0495

IMPROVEMENT IN HEALTH-RELATED QUALITY OF LIFE (HRQOL) WITH LONG-TERM ELTROMBOPAG TREATMENT IN ADULTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (ITP): RESULTS OF EXTEND FROM JUNE 2006 TO FEBRUARY 2011

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Background. Eltrombopag is an oral, nonpeptide, thrombopoietin receptor agonist approved for treatment of chronic immune thrombocytopenia (cITP). EXTEND is an ongoing, open-label extension study of long-term cITP treatment with eltrombopag in patients completing a prior eltrombopag study (including 6-month follow-up). Interim EXTEND data describing effects of eltrombopag on health-related quality of life (HRQoL) from June 2006-December 2008 have been reported (Bussel 2009); effects of longer treatment on HRQoL have not been reported. **Aims.** To assess longer-term effects of eltrombopag on HRQoL among cITP patients in EXTEND from June 2006 to February 2011, following a median treatment duration >2 years. **Methods.** HRQoL (7-day recall) was self-reported at baseline (BL) and upon progression toward establishing an effective dose, using the SF-36v2, FACIT-Fatigue subscale, MEI-SF, and 6 items from FACT-Thrombocytopenia (FACT-Th6), focusing on bruising and bleeding concerns and limitations in daily physical and social activities. Generalized estimating equations were used to account for multiple assessments of the same patient. Clinical meaningfulness was expressed using developer specifications or minimally important differences (MIDs) using a distribution-based method (0.5 standard deviations of BL scores). **Results.** As of this analysis, 287-291/300-301 patients in EXTEND (depending on the assessment tool) had both BL and on-therapy HRQoL assessments. BL SF-36v2 and FACIT-Fatigue scores were consistent with shorter-term eltrombopag studies, reflecting impaired HRQoL in chronic ITP patients compared with the general US population (Ware 2000; Cella 2002). Improvements from BL in HRQoL were observed throughout EXTEND while remaining lower than US general population norms.Patients experienced statistically significant (*P*<0.05) and modest clinically meaningful improvements from BL at every stage of EXTEND for the FACIT-Fatigue and FACT-Th6, and during Stages 2-4 for the MEI-SF, corresponding to reductions in fatigue and bleeding/bruising concerns and improved motivation and energy (Table 1).

Table. Adjusted HRQoL Score Changes From BL During EXTEND. Covariates included time since BL visit; stage; prior splenectomy; BL platelet count «15x10^9/µL; age; ethnicity; body mass index; region; sex; country; prior exposure to eltrombopag; and treatment termination status. Significant values (*P*<0.05) are highlighted.

HRQoL Measure	BL Score (95% CI)	Dosing Identification (Stage 1)[a]	Minimizing ITP Medications (Stage 2)[b]	Optimizing Dosing (Stage 3)[c]	Maintenance Dosing (Stage 4)[d]
		Estimated Change From BL (95% CI)			
FACIT-Fatigue	35.6 (34.5-36.8)	1.5 (0.2-2.8) *P*=0.024	2.5 (0.6-4.3) *P*=0.010	2.3 (1.1-3.5) *P*<0.001	2.6 (1.3-3.9) *P*<0.001
FACT-Th6	14.6 (14.0-15.2)	1.5 (0.9-2.2) *P*<0.001	2.2 (1.3-3.1) *P*<0.001	2.1 (1.5-2.7) *P*<0.001	2.1 (1.5-2.8) *P*<0.001
MEI-SF	70.0 (67.8-72.1)	2.4 (0.0-4.7) *P*=0.053	4.3 (0.6-7.9) *P*=0.024	3.0 (0.7-5.2) *P*=0.011	3.3 (0.8-5.8) *P*=0.009
SF-36v2 PCS	46.1 (45.3-47.0)	1.1 (0.4-1.9) *P*=0.004	1.0 (-0.2-2.3) *P*=0.106	1.4 (0.6-2.2) *P*=0.001	2.1 (1.2-3.1) *P*<0.001
Physical functioning domain	73.5 (71.0-75.9)	3.2 (0.9-5.5) *P*=0.006	2.2 (-0.8-5.1) *P*=0.151	4.2 (1.9-6.5) *P*<0.001	5.1 (2.6-7.7) *P*<0.001
Physical role domain	67.3 (64.6-69.9)	4.2 (1.2-7.3) *P*=0.007	4.3 (-0.3-8.8) *P*=0.069	3.1 (0.2-6.0) *P*=0.035	4.6 (1.4-7.8) *P*=0.005
Bodily pain domain	72.8 (70.2-75.4)	2.1 (-0.7-4.9) *P*=0.140	0.9 (-4.3-6.1) *P*=0.737	0.1 (-3.1-3.3) *P*=0.951	3.6 (0.4-6.8) *P*=0.028
General health domain	52.5 (50.4-54.7)	1.4 (-0.6-3.4) *P*=0.185	3.7 (0.4-7.0) *P*=0.029	4.0 (2.0-6.0) *P*<0.001	5.2 (2.8-7.6) *P*<0.001
SF-36v2 MCS	45.4 (44.3-46.5)	1.2 (-0.1-2.6) *P*=0.063	1.7 (-0.3-3.8) *P*=0.099	0.8 (-0.4-2.0) *P*=0.215	1.0 (-0.5-2.5) *P*=0.177
Vitality domain	54.4 (52.0-56.7)	3.0 (-0.2-6.2) *P*=0.064	5.7 (2.0-9.4) *P*=0.002	3.5 (1.0-6.1) *P*=0.007	4.3 (1.1-7.5) *P*=0.009
Social functioning domain	74.6 (72.1-77.1)	4.1 (1.5-6.7) *P*=0.002	3.6 (-0.6-7.8) *P*=0.094	3.5 (0.7-6.3) *P*=0.013	3.5 (0.3-6.7) *P*=0.032
Emotional role domain	74.1 (71.6-76.7)	2.3 (-0.7-5.3) *P*=0.127	1.8 (-2.6-6.1) *P*=0.426	1.3 (-1.6-4.2) *P*=0.387	2.3 (-0.9-5.4) *P*=0.158
Mental health domain	68.1 (66.1-70.1)	2.2 (0.0-4.5) *P*=0.054	3.2 (-0.4-6.7) *P*=0.078	1.3 (-0.9-3.5) *P*=0.232	2.5 (0.0-5.0) *P*=0.048

[a]Identification of an eltrombopag dose that increases platelets ≥100,000/µL.
[b]Reduction or elimination of any concomitant ITP medication use while maintaining platelets ≥50,000/µL.
[c]Identification of minimal eltrombopag dose to maintain platelets ≥50,000/µL in conjunction with the minimal dose of any concomitant ITP medication use.
[d]Long-term treatment and monitoring using doses identified in Stage 3.
BL, baseline; CI, confidence interval; ITP, immune thrombocytopenia; MCS, mental component summary; PCS, physical component summary.

Statistically significant improvements from BL for the SF-36v2 physical component summary (PCS) were reported during eltrombopag dose identification (Stage 1), optimization (Stage 3), and maintenance (Stage 4; Table 1). No such improvements from BL were reported for the SF-36v2 mental component summary (MCS). Statistically significant improvements from BL occurred for several SF-36v2 domains, notably when patients reached maintenance dosing. Improvements in bodily pain and mental health domains were limited to the maintenance dosing stage.Patients withdrawing from or completing study reported mean HRQoL scores at their last on treatment visit that were at or slightly lower than BL, a decrease driven by withdrawals (41%); in contrast, HRQoL remained higher than BL for patients remaining on treatment. **Summary and Conclusions.** EXTEND study results suggest that treatment with eltrombopag is associated with improvement in HRQoL across multiple measures and domains related to fatigue and physical dimensions of HRQoL. Improvements were seen across multiple stages while on therapy, especially during the maintenance stage, while consistently remaining lower than general population norms. Emotional role, mental health, and bodily pain improvements were limited, suggesting difficult domains to modify even with extended therapy. These results support interim findings reported previously, suggesting durable improvements in HRQoL domains, especially fatigue and physical functioning, in cITP patients with long-term eltrombopag treatment.

0496

CONNECTIVE TISSUE METABOLITES AND CYTOKINES IN IMMUNE THROMBOCYTOPENIA (ITP) TREATED WITH THROMBOPOIETIN RECEPTOR AGONISTS (TPO-RA)

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Background. TPO-RAs increase platelet counts in patients with ITP by promoting magakaryocyte proliferation and differentiation. One potential adverse effect of TPO-RA is the stimulation of bone marrow fibrosis (BMF). The relation between megakaryocyte stimulation and BMF has been demonstrated in animal models by showing that high doses of TPO-RA result in dose-dependent BMF - a process that may be mediated by transforming growth factor-β (TGF-β). However, in humans the knowledge regarding the extent of BMF with prolonged exposure to TPO-RA is limited.The N- and C-terminal propeptides of collagen I and III are removed in a 1:1 ratio before fibril formation occurs- the basis for their use as biomarkers for collagen production in fibroproliferative conditions. Hyaluronan is abundantly present in the extracellular matrix, particularly at sites of tissue inflammation. Altered concentration of these substances has been reported in patients with primary myelofibrosis.**Aims**The aims of the study were to determine blood levels of connective tissue metabolites and fibrosis-related growth factors and their significance in consecutive samples collected from ITP patients treated with TPO-RA. **Methods.** Forty-eight TPO-RA treated ITP patients in whom BM biopsies were performed during treatment were identified. Pre-treatment (<2 months) and sequential on-treatment frozen blood samples were retrieved at 6 months intervals, whenever available. Myelofibrosis (MF) was graded according to the European Consensus Grading System. Connective tissue metabolites were analyzed in serum by radioimmunoassay; TGF-β was analyzed in plasma by ELISA and Hepatocyte GF (HGF) by immuno-bead-based multiplex assay (R&D systems). **Results.** Median age (28 females 58%) was 50 years. Median duration of treatment with TPO-RA to the time of last available BM Biopsy (BMB) was 1.8 years (range 0.15-6.7). In 46 evaluable biopsies, reticulin was graded MF-0 in 8 (17%), MF-1 in 31 (67%), MF-2 in 7 (15%). Median duration of exposure was 1.8 years in MF0/1 and 1.5 years in MF-2. Median values of collagen metabolites and GFs are shown in the Table. **Discussion and Conclusions**85% of the patients treated with TPO-RA had no or low-grade (MF-1) reticulin deposition in the BM. No difference in duration of treatment was noted between those with less than MF-2 and those with MF-2. Sequential measurements of connective tissue metabolites revealed decreasing levels of PINP and PIIINP over time, confirming our previous findings, and suggesting decelerated collagen synthesis despite sustained platelet response, and unchanged dosage. TGF-β increased initially after treatment, but then declined gradually along with PIIINP. HGF, an antifibrotic and angiogenesis-related GF, seemed to decrease after initiation of TPO-RA and remained low.The number of samples limits subgroup analysis, however, levels of HA, ICTP and HGF at 24 months appeared higher in MF-2 than MF-0/1.In conclusion, persistent low-grade reticulin fibrosis was observed in most patients; MF-2 was seen in several, but none had MF-3. Biomarkers of collagen synthesis and fibrosis-related-GFs tended to decrease while collagen degradation remained steadily elevated. These findings suggest that ITP is associated with an abnormal bone marrow stromal reaction, which is partially corrected during TPO-RA treatment.

Table 1. Median concentrations of collagen metabolites and growth factors in thrombopoietin-treated ITP patients.

	-2 - 0 m n=29	6 m n=34	12 m n=34	18 m n=21	24 m n=18	*p*-value †	MF-<2 n=15	MF-2 n=3
Plt count x10^9/L	24	82	94	103	62	NS	72	39
PIIINP ug/L	6.2	6.9	6.6	5.5	5.0	NS	5.0	5.2
PINP ug/L	53.3	53.8	56	44*	46**	*0.027 **0.07	47	42
HA ng/mL	39	29	26	38	28	NS	23	38
ICTP ug/L	6.4	6.6	6.4	4.6	5.6	NS	5.4	7.0
TGF-B pg/mL	891	2157*	1452	1836	1028	*0.012	1098	1028
HGF pg/mL	1712	851*	622**	661**	1163 ***	*0.02 **0.005 ***0.01	1070	1332

†All other comparison between pre-treatment and on-treatment were statistically not significant (NS)
NP= not performed; m= month

0497

EFFECT OF THROMBOPOIETIN-RECEPTOR AGONISTS ON CIRCULATING CYTOKINE AND CHEMOKINE LEVELS IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Background. Thrombopoietin receptor agonists (TPO-RA) are new treatment modalities for chronic ITP, exerting their effect by stimulating platelet production. It is well established that platelets are associated with inflammatory processes involving both the innate and the adaptive immune compartment, and preliminary studies show that treatment with TPO-RA increase suppressive activity of regulatory T-cells. The effect of TPO-RA on inflammatory cytokine production in ITP patients before and during treatment has not been investigated previously. **Aims.** To determine the influence of TPO-RA on the production of a panel of inflammatory cytokines and chemokines in chronic ITP patients before and during treatment with TPO-RA compared to healthy controls. **Methods.** Cytokines and chemokines in EDTA-plasma samples from 48 ITP patients treated with TPO-RA (28 females, median age 50 years [IQR 20-69 years], median platelet counts 24 x 10^9/L [IQR 15-47x 10^9/L]) and 16 healthy controls (9 females, median age 37 years [IQR 22-51 years]) were analysed by enzyme-linked immunosorbent assay and immuno-bead-based multiplex assay. **Results.** Compared to healthy controls, elevated levels of the pro-inflammatory immunomodulating molecules TNF-a ($p < 0.001$) and IP-10 ($p < 0.001$) were observed in pre-treatment samples from ITP patients, as were levels of the anti-inflammatory anti-osteolytic molecule osteoprotegerin (OPG) ($p < 0.05$). During treatment with TPO-RA, levels of IP-10 (after 18 months, $p < 0.05$) and OPG (after 12 and 18 months, $p < 0.05$) decreased compared to pre-treatment values, while TNF-a remained elevated. The IL-1ra/IL-1b ratio decreased after 6 months of treatment ($p < 0.05$), although at no point were levels statistically

different from those of healthy controls. Levels of CD40L increased after 6 months of treatment ($p < 0.05$) but then decreased to pre-treatment values that were comparable to those of healthy controls. IFN-g and IL-10 did not differ significantly from healthy controls or change during treatment. **Summary and Conclusions.** It is well established that platelets possess immunomodulatory properties. Since TPO-RA increase platelet counts by stimulating platelet production in the bone marrow, the question arises of whether this treatment also exerts some effects on the immune system. In this study we report, for the first time, the influence of TPO-RA on a panel of immunolmodulatory cytokines and chemokines, compared to both pre-treatment values and healthy controls. We found a decrease in concentrations of the pro-inflammatory mediators IP-10 and CD40L, accompanied by a decrease in the anti-inflammatory osteoprotegerin as well as an initial decrease in the IL-1ra/IL-1b ratio during treatment. However, levels of TNF-a remained elevated indicating that autoreactive T-helper cells stay activated at least to some extent during treatment. Levels of IL-10 and IFN-g did not differ from healthy controls or change during treatment. Our findings suggest that TPO treatment influences the expression of immunomodulatory signalling molecules associated with the bone marrow micro-environment. The underlying effector mechanisms and clinical implications of these findings need to be further investigated.

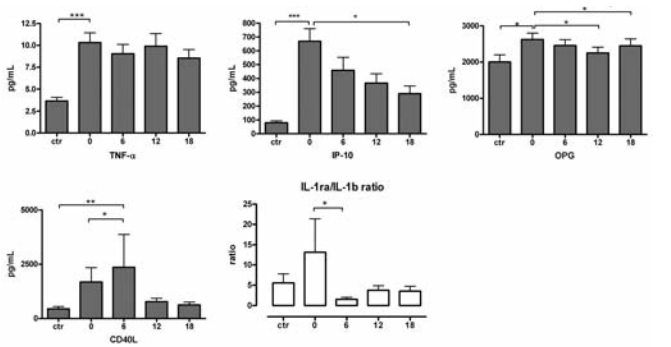

Figure 1. Cytokine and chemokine levels (mean, SEM).

0498

EFFECT OF ACTIVATED PLATELETS ON INTERLEUKIN-10 PRODUCTION BY MONOCYTES IN VITRO

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Background. Platelets are associated with inflammatory processes involving both the innate and the adaptive immune compartment. Recent studies have indicated that activated platelets may stimulate maturation of monocyte-derived dendritic cells and regulate T- and B-cell activity, but little is known about the direct effects of platelets on monocytes. **Aims.** To investigate the effect of activated autologous platelets on cytokine production and $CD4^+$ T-cell proliferation in cultured mononuclear cells. **Methods.** Mononuclear cells (MNCs) from 11 healthy donors were stimulated with the self-antigen thyreoglobulin (TG) and the foreign antigens tetanus toxoid (TT) and lipopolysaccharide (LPS) and cultured in the absence or presence of activated autologous platelets. Supernatants were harvested after 24 hours and analysed for Th1/Th2 cytokine production, $CD4^+$ T-cell proliferation was determined by flowcytometry as dilution of carboxyfluorescein succinimidyl ester (CFSE). MNCs from an additional 10 healthy donors were stimulated with TT or TG and cultured correspondingly. After 18 hours of culture monocytes were analysed for IL-10 and TNF-a secretion by flow cytometry. **Results.** Addition of activated platelets caused an increased production of IL-10 in MNC cultures stimulated with LPS ($p < 0.001$) and TG ($p < 0.05$). Moreover, activated platelets reduced the production of TNF-α in cultures stimulated with LPS ($p < 0.001$) and TG ($p < 0.05$) and inhibited the production of IL-6 in LPS-stimulated cultures ($p < 0.01$). Correspondingly, addition of activated platelets increased the proportion of monocytes secreting high amounts of IL-10 following stimulation with TG ($p < 0.05$. Platelet/monocyte aggregates were detected flow cytometrically as events double-positive for the monocyte marker CD14 and the platelet marker CD61. Platelet/monocyte aggregates were found in the cultures irrespective of platelets had been added *in vitro*, but their proportion was increased after addition of activated platelets to cultures stimulated with TG (3.9 vs 8.0 %, $p < 0.01$). Interestingly, platelet-bearing monocytes showed significantly higher IL-10 secretion than monocytes without adherent plateles in MNC cultures stimulated with TG ($p < 0.05$). Moreover, the presence of activated platelets inhibited the proliferation of $CD4^+$ T cells elicited by TT ($p < 0.05$) or TG ($p < 0.01$). **Summary and Conclusions.** The data suggest that activated platelets exert an immunoregulatory function by enhancing IL-10 production by monocytes and that this, at least in part, depends on direct platelet-monocyte adhesion. Platelets inhibit proliferative T helper-cell responses to self- and non-self antigens, probably via their induction of monocyte IL-10 production.

0499

PLATELET FLOW CYTOMETRY AND MARKERS OF IMMUNE ACTIVATION IN ASYMPTOMATIC, TREATMENT- NAÏVE HIV INFECTION

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Background. In the era of antiretroviral therapy (ART), the risk of acquired immune deficiency syndrome (AIDS)-related deaths has decreased and people living with Human Immunodeficiency Virus (HIV) have prolonged life spans. HIV-infected patients are now at a higher risk of developing cardiovascular disease (CVD) and other inflammatory-associated complications. Activated platelets play a key role during infection and the inflammatory process, by mediating interactions between cells of innate immunity and the endothelium. Soluble markers of platelet activation are increased during HIV-infection. P-selectin (CD62P) is expressed on the platelet surface only upon platelet activation. Activated platelets play a role in HIV-induced atherosclerosis through the expression and release of mediators that induce endothelial activation and support leukocyte adhesion to the inflamed vessel wall. **Aims.** To determine the levels of platelet activation and function by Flow Cytometry in HIV-infected individuals as compared with uninfected controls; and further, to correlate these levels with full blood count, coagulation and other inflammatory parameters. **Methods.** In this study, a total of 57 adult South Africans were recruited from a clinic in the Western Cape. These included 32 HIV positive, ART- naïve patients and 25 HIV negative individuals. Platelet activation and platelet function were investigated using a novel whole blood Flow Cytometry assay: Platelet-specific markers CD41a and CD42b were used to ensure platelet-specific gating. CD62P expression was used to evaluate platelet activation. Platelet function was evaluated by investigating the response of platelets to endogenous agonists; adenosine diphosphate(ADP) and arachidonic acid (AA) at varying concentrations. A full blood count and differential as well as fibrinogen levels were determined using routine hematology laboratory analyzers. **Results.** Baseline levels of CD62P expression were significantly higher in treatment-naïve HIV positive patients compared with uninfected controls (mean %CD62P 71.74 + 2.18 vs control 54.52+ 2.42; p=<0.0001). CD62P expression correlated directly with platelet counts (r=0.374, p=0.042) whereas platelet counts showed an inverse correlation with viral loads (r=-0.65, p=0.0061). Fibrinogen levels correlated with absolute WCCs (r=0.659, p=0.0021); absolute neutrophil count (r=0.619, p=0.0105); absolute monocyte count (0.562, p=0.0235) and hsCRP (r=0.688 p=0.0011). Importantly, fibrinogen showed a negative correlation with CD4 counts (r=-0.594, p=0.0014) and may be a valuable marker of prognosis in treatment- naïve, HIV positive patients. HsCRP levels correlated with absolute neutrophil counts (r=0.392, p=0.0005).The HIV Group showed an overall hyper-response to ADP at a concentration 0.025 μM as compared to uninfected controls (62.34 + 9.7 vs control 36.90 + 5.7, p=0.0433). **Conclusions.** We describe a novel Flow Cytometry technique that may be used to evaluate the levels of platelet activation and function in HIV-infected patients. In addition we report a cost-effective panel in the form of fibrinogen, WCC and platelets that may be valuable in predicting the progression to AIDS or other inflammatory- associated complications in treatment-naïve HIV-infected patients. This may impact on patient management prior to initiation of ART and provide a tool for monitoring responses to treatment. Longitudinal studies will be required to assess risk of thrombosis based on these assays and the potential for application in other chronic inflammatory conditions.

0500

SPLENECTOMY IN IMMUNE THROMBOCYTOPENIA: RESULTS OF 214 ITP PATIENTS WITH A MINIMUM FOLLOW-UP OF 10 YEARS

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Background. Splenectomy is still regarded as the best second line treatment of immune thrombocytopenia (ITP). Recently, new medical therapies (anti-CD20 monoclonal antibodies and thrombopoietin mimetics) have entered into clinical practice, encouraging a general tendency to delay splenectomy. Consequently, the importance to define the efficacy and safety of splenectomy in the long-term is substantial. **Patients and Methods.** We retrospectively analyzed clinical data of 214 ITP patients who underwent splenectomy between 1962 and2001, in 6 different European hematological Institutions and have now a minimum follow-up of 10 years from surgery (median, 20.4 years; range 10-43 years). **Results.** Overall, 140/214 (65%) ITP patients were women. Median time from diagnosis to splenectomy was 13 months (range 0-254 months) and median age at splenectomy was 33 years (range 6-74 years). All patients but 3 underwent splenectomy after one or more steroid-based treatments. Overall, 187 patients (87%) achieved a response (R, platelet count >30x10^9/L), which was complete (CR, platelet count >100x10^9/L) in 162 patients (76%). In 123 cases (66%), the response was stable at last contact. Sixty-four patients (34%) lost the response during the follow-up. Sixteen of these patients (25%) experienced an early relapse (within 6 months from splenectomy), while in the remaining 48 patients, relapse occurred after a median time of 25 months (range, 6-256), for a relapse-free survival of 65% at 20 years of all responding patients (Figure 1). Median time to relapse was significantly shorter in R-patients compared to CR-patients (12 vs 21 months, p<0.01). Overall, 90 patients of 214 patients (42%) needed further treatment after surgery, that induced at least a R in 77 patients (86%). Fifty-five patients (26%) experienced 204 hemorrhagic events (14 of which grade 3-4 WHO), after a median time of 15 months. Twenty-six thrombotic events (4 fatal) have been observed in 18 patients (8%), after a median time of 200 months (range 0-392 months). Seventy patients (33%) experienced one or more infectious episodes after surgery, which were severe (pulmonary) in 33 cases and were observed after a median follow-up of 36 months (range 0-355 months). Forty-one of them (59%) were vaccinated against encapsulated bacteria and 38 (54%) received one or more treatments because of refractory or relapsed disease after splenectomy. Thirty-five patients died during follow-up (median age 73 years at death, range 43-88 years). The causes of death were related to ITP or ITP-treatment in 3 cases (2 patients had fatal hemorrhages, 1 patient sepsis). **Conclusions.** Splenectomy induced a stable remission in 66% of ITP patients with a minimum and median follow-up after splenectomy of 10 and 20.4 years respectively. Relapses were mainly observed during the first months following surgery, reaching a plateau after about 12 years (Figure 1). The incidence of late severe infectious complications was not negligible, probably due to the fact that about 40% of these patients did not receive prophylactic vaccinations and half of them have needed further medical therapy. A detailed analysis concerning prognostic factors for response after splenectomy is also planned.

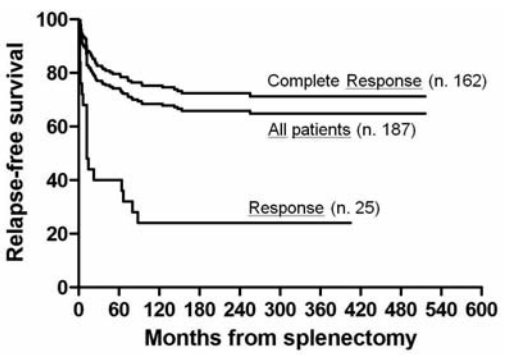

Figure 1.

0501

ELTROMBOPAG FOR THE TREATMENT OF CHILDHOOD CHRONIC ITP: THE PETIT STUDY, A PLACEBO-CONTROLLED CLINICAL TRIAL

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Background. Most therapies for chronic immune thrombocytopenia (cITP) have side effects that reduce their tolerability in children. PETIT is an ongoing open-label and randomized, placebo-controlled trial to investigate efficacy and safety of eltrombopag in children with cITP who failed initial therapy and have platelets <30,000/μL. **Aims.** To report open-label data from PETIT. **Methods.** Five patients were enrolled in Cohort 1 (12-17 years) and received open-label eltrombopag. After 12 weeks, safety, pharmacokinetics, and platelet counts were reviewed and 18 patients (not part of this abstract) were randomized 2:1 to eltrombopag or placebo. All patients received 24 weeks of eltrombopag therapy. In Cohort 1, the 12-week review identified no safety issues, so dosing began for 5 open-label patients in Cohort 2 (6-11 years), with identical procedures followed for safety review and randomization of 18 patients. Cohort 3 (1-5 years) opened once data from the completed randomized periods of Cohorts 1 and 2 were evaluated by an independent data safety monitoring board.Conservative starting doses were used (Cohort 1, 25 mg daily; Cohort 2, 25 mg [weight ≥27 kg] or 12.5 mg [<27 kg]; Cohort 3, 0.7 mg/kg) and adjusted as needed to a maximum of 75 mg daily, to maintain platelet count ≥50,000-<400,000/μL. Starting doses were reduced 50% for East Asian patients. Assessments were weekly until a stable dose was achieved and monthly thereafter. Response was platelets ≥50,000/μL at any time in the absence of rescue treatment. **Results.** Data from the 15 open-label patients, 5 from each cohort, are presented (Table 1). At baseline, 9 (60%) patients had platelets ≤15,000/μL, 1 was splenectomized, and 1 was receiving a stable dose of methylprednisolone.Median doses during 24 weeks were 75 mg for both Cohorts 1 and 2, and 70 mg for Cohort 3. All patients completed 24 weeks of treatment.Response was achieved at least once by 80% of patients (4/5 in each cohort). Between weeks 17-24, 8/15 (53%) patients had platelets ≥50,000/μL in ≥50% assessments and 11/15 (73%) had ≥30,000/μL in 80% of assessments, without rescue treatment. The patient receiving methylprednisolone discontinued it after 71 days. Seven (47%) patients received rescue treatment.Incidence of WHO bleeding grades 1-4 and 2-4 was 87% (13/15) and 33% (5/15) at baseline, decreasing to 50% (7/14) and 7% (1/14) by Week 24.All patients reported adverse events (AEs). 88% were grades 1-2, 9% were not graded, and there was 1 grade 4 AE (neutropenia). Serious AEs were reported for 2 patients (neutropenia, epistaxis). The most common AEs were headache (8 patients, 53%), vomiting (7, 47%) and diarrhea (5, 33%). No patient had AEs of liver enzyme elevation or thrombosis, and none were withdrawn due to AEs.**Summary and Conclusions.** Preliminary data suggest that eltrombopag is safe and potentially effective for children with cITP failing first-line therapy. No safety issues prevented enrollment into subsequent cohorts or the double-blind phase. Efficacy conclusions are limited by small patient numbers, lack of comparator arm, and low starting doses. The ongoing randomized study will further characterize safety and efficacy of eltrombopag in childhood cITP.

Table 1. Demographics and baseline characteristics.

	Cohort 1 n=5	Cohort 2 n=5	Cohort 3 n=5	Total N=15
Median age, years (range)	14 (13-16)	10 (8-11)	4 (2-5)	10 (2-16)
Sex, n				
Male	2	3	2	7
Female	3	2	3	8
Ethnic background, n				
Caucasian	2	4	3	9
African American	1	0	1	2
Central/South Asian (CSA)	1	1	0	2
Unknown	1	0	0	1
Mixed race–CSA/Caucasian	0	0	1	1
Median platelets, x10^3/μL (range)	9 (1-23)	17 (7-21)	15 (4-19)	13 (1-23)
Median duration of ITP, years (range)	2.3 (1-11)	2.6 (0.5-7.9)	1.2 (0.6-2.9)	1.5 (0.5-11)
Median number of prior ITP therapies (range)	2 (1-4)	2 (1-5)	3 (2-6)	3 (1-6)

0502

MEASUREMENT OF SERUM B-CELL ACTIVATING FACTOR OF TUMOR NECROSIS FACTOR FAMILY (BAFF) IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA AND ITS CORRELATION WITH IMMUNOSUPPRESSIVE THERAPY

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Background. B-cell activating factor of the tumor necrosis factor family (BAFF) is a homotrimeric type 2 transmembrane protein that also exists in a soluble form. It belongs to the family of tumor necrosis factor (TNF) ligands. BAFF is expressed at the surfaces of myeloid cells and antigen- presenting cells (APCs) and induces B-lymphocyte proliferation and immunoglobulin secretion. **Aims.** to assess serum BAFF level in patients with idiopathic thrombocytopenic purpura and correlate its level with response to immunosuppressive therapy (corticosteroids). **Methods.** The study included 60 subjects; 40 patients with newly diagnosed idiopathic thrombocytopenic purpura (ITP) who were followed for 3 months after immunosuppressive therapy (steroids) then divided into responders and non responders and 20 healthy control subjects. **Results.** Serum BAFF level was lowest among controls (mean ± SD 3.4+2.2 ng/mL) ITP patients (responders and non-responders) before treatment (mean ± SD19.8+2.9 ng/ml and 20.5+8.8 ng/ml respectively) with statistically highly significant difference (P value is highly significant <0.001), But no significant difference between responders and non responders. On the other hand BAFF level after treatment was still high among non responder group (mean ± SD 18+7.6 ng/ml) with statistically highly significant difference in comparison to responders and controls (mean ± 3.6+1.9 and 3.4+2.2 ng/mL respectively), with highly significant P value (<0.001). **Conclusions.** It was found that BAFF decreased markedly among total cases and among responder group with statistically highly significant difference. Percentage of change was 84% among responders compared to 10% only among non responders.Therefore,it is possible that transcriptional down regulation of BAFF represents a novel target in therapy of ITP.

0503

COST-EFFECTIVENESS OF ROMIPLOSTIM FOR THE TREATMENT OF ADULT IMMUNE THROMBOCYTOPENIA IN IRELAND

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Background. Immune thrombocytopenia (ITP) is an autoimmune disorder that results in accelerated platelet destruction as well as reduced platelet production. The use of thrombopoietin-receptor agonists romiplostim and eltrombopag are recommended for second-line use in adult ITP patients[1]. **Aims.** To assess the cost-effectiveness of treatment of ITP in Irish patients by firstly introducing romiplostim versus eltrombopag after rituximab treatment into current standard of care (SC; including rituximab) for adult ITP in Ireland, and secondly placing romiplostim in different positions in the treatment pathway. **Methods.** A lifetime treatment-sequence cost-utility model was built from an Irish healthcare payer perspective. The model was driven by platelet response (platelet count ≥50×10^9/L), which determined treatment progression, need for rescue therapy and risk of experiencing bleeds. Clinical inputs, patient characteristics and utilities were derived from indirect comparison of efficacy between romiplostim and eltrombopag[2], clinical trials, International Consensus Guidelines[1] and other published evidence. Treatment of bleed-related events consisted of rescue therapy (intravenous immunoglobulin IVIg and intravenous steroids), physician visits and hospitalizations associated with bleeds. Treatment sequences and health care utilization practice was validated by clinical experts. Costs were derived from published Irish reimbursement lists and other relevant sources. For different treatment sequences, which included romiplostim, eltrombopag and rituximab, total treatment costs and Quality Adjusted Life Years (QALYs, One QALY = one year in full health) over a patient's lifetime were calculated. **Results.** In the treatment sequence after rituximab, compared to eltrombopag, romiplostim was associated with cost savings of €25,386 and increased QALYs by 0.96 over a patient's lifetime. Cost savings are due to a reduction in the costs of rescue therapies (IVIg and steroids) and a reduction in the cost of treating bleeds. In the same sequence, compared to SC including rituximab, romiplostim was associated with cost savings of €27,022 with an increase of 1.14 QALYs over a patient's lifetime (Table 1). When romiplostim and eltrombopag were placed before rituximab in the treatment sequence, use of romiplostim remained the more efficacious treatment pathway, at a lower cost compared to both eltrombopag and SC. **Summary.** Romiplostim not only improves clinical outcomes for patients with ITP in Ireland but, by reducing bleeding-related events and the use of rescue therapies, also results in cost savings and improved quality of life when compared to current treatment practices. **References**1. Provan D. Blood 2010, 115:168 - 186.2. Thrombocytopenic purpura - eltrombopag: response to the GlaxoSmithKline comments on the appraisal consultationdocument and Evidence Review Group report.http://www.nice.org.uk/guidance/index.jsp?action=download&o=50715. Last accessed January 5, 2012.

Table 1. Cost effectiveness of romiplostim compared to eltrombopag and SC, including rituximab in different treatment pathways.

Treatment Sequence with Romiplostim after Rituximab Treatment		Treatment Sequence with Eltrombopag after Rituximab Treatment		Treatment Sequence with Standard of Care, including Rituximab	
Treatment	*% Receiving Treatment **	*Treatment*	*% Receiving Treatment **	*Treatment*	*% Receiving Treatment **
1. Rituximab	80%	1. Rituximab	80%	1. Rituximab	80%
2. Romiplostim	100%	2. Eltrombopag	100%	2. Azathioprine	59%
3. Azathioprine	59%	3. Azathioprine	59%	3. Mycophenolate mofetil	37%
4. Mycophenolate mofetil	37%	4. Mycophenolate mofetil	37%	4. Cyclosporine	4%
5. Cyclosporine	4%	5. Cyclosporine	4%		
Cost Saving Associated with Romiplostim		**€25,386**		**€27,022**	
QALYs Gained by Treatment with Romiplostim		0.96		1.14	
ICER		**Dominant**		**Dominant**	
Treatment Sequence with Romiplostim before Rituximab Treatment		**Treatment Sequence with Eltrombopag before Rituximab Treatment**		**Treatment Sequence with Standard of Care, including Rituximab**	
Treatment	*% Receiving Treatment **	*Treatment*	*% Receiving Treatment **	*Treatment*	*% Receiving Treatment **
1. Romiplostim	100%	1. Eltrombopag	100%	1. Rituximab	80%
2. Rituximab	80%	2. Rituximab	80%	2. Azathioprine	59%
3. Azathioprine	59%	3. Azathioprine	59%	3. Mycophenolate mofetil	37%
4. Mycophenolate mofetil	37%	4. Mycophenolate mofetil	37%	4. Cyclosporine	4%
5. Cyclosporine	4%	5. Cyclosporine	4%		
Cost Saving Associated with Romiplostim		**€21,829**		**€22,673**	
QALYs Gained by Treatment with Romiplostim		0.99		1.17	
ICER		**Dominant**		**Dominant**	

ICER = incremental cost effectiveness ratio
QALYs = quality adjusted life years. One QALY = one year in full health
Dominant = More effective at lower cost
* the proportion of patients receiving the next treatment if preceding treatments fail. O'Keeffe D. Midwestern Regional Hospital, Limerick, Ireland. Data on file

0504

ALPS AND ALPS- RELATED DISORDERS. WHAT THE HAEMATOLOGISTS HAVE TO FACE WITH : SINGLE CENTER EXPERIENCE

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Background. Autoimmune lymphoprolipherative syndrome (ALPS) is a disease characterized by lymphadenopathy, hepatosplenomegaly, and autoimmune cytopenias, such as haemolytic anemia, neutropenia and thrombocytopenia. According to current diagnostic criteria (table I), "ALPS related disorders"shares the lymphoproliferation and the apoptotic defects characteristic of ALPS , but do not fullfill at all the above citated criteria. **Aims.** to describe a cohort of patients referred to our center from 1981 to 2011 for recurrent/refractory cytopenias and/or lymphoproliferation who were subsequently defined and treated as ALPS and ALPS related-disorders. **Methods.** patients with refractory autoimmune haemolytic anemia, chronic thrombocytopenia, persistent neutropenia and/or lymphoproliferation entered the analysis. ALPS and "ALPS related disorders"as from literature (table I) .Patients data were obtained throught medical records. Anagraphic, clinical and imaging data, biochemical tests, double-negative T (DNT) cells test (pathological if > 1.5% of total lymphocytes), FAS mediated apoptosis test and molecular analysis when available, autoimmunity markers (DAT , IAT, ANA, indirect antibodies against neutrophils and platelets) were collected. **Results.** 38 patients, 20 males and 18 females entered the study, median age at presentation was 6.9 years (0.1-15years). ALPS criteria were fully matched in 9pts/38 (24%), the remaining 29pts (76%) were classified as ALPS-related disorders. Recurrent/refractory thrombocytopenia in 15/38 (40%), acute refractory haemolytic anemia in 10/38 (26%), lymphoproliferation in 9 pts/38 (24 %) and neutropenia in 4/38 (10 %) was the leading symptom. In 5 patients cytopenia was bilinear and 1 trilinear. At least one autoimmunity marker was positive in two third of the cohort and. DAT was the most frequent althoughnot necessarily associated to anemia. Mutations of TNFR6 gene, studied in 13/38 patients, were found in 2 (ongoing in 5). In 27/38 pts (71%) treatment was necessary. The 11 patients who did not require treat-

ment were affected by "non sintomatic lymphoproliferation", mild throrombocytopenia and two patients with neutropenia were managed with G-CSF only. First line treatment was steroids providing complete response in 11/27 (37%) of cases (in all but one it was stopped). One patient died because of hyperacute haemolytic process in a time when "new drugs"were not available yet. In the remaining 15 patient a second (7/15, 50%), third (5/15, 33%), fourth line regimen (3/15-17%) was needed. "Second line drugs"were mainly cyclosporin in combination with steroids (4 pts: transient response), mycophenolate mofetil (9 pts: 4 pts transient and 5complete response) and sirolimus alone (5 pts: 4 complete response and 1 in progress). **Summary and Conclusions.** Diagnosis of ALPS andALPS related diseases has to be considered in patients affected with chronic thrombocytopenia, refractory autoimmune haemolytic anemia and chronic neutropenia associated with lymphoproliferation and autoimmunity markers. Double negative T cells , a required criteria of ALPS, was detectable only in one fourth of the population. Treatment was necessary in more than 2/3 of patients and in nearly half of them more than "one line regimen" was needed. In our experience, mycophenolate mofetil and sirolimus are efficacious to control the diseases sparing long term steroids use with their well-known side effects.

Table 1. Revised classification of ALPS and related disorders. Oliveira JB *et al*. Blood 2010.

ALPS		
Revised nomenclature	**Gene**	**Definition**
ALPS-FAS	*FAS*	Germ-line homozygous or heterozygous *FAS* mutations. Previously ALPS type 0 and type Ia.
ALPS-sFAS	*FAS*	Somatic mutations in *FAS*. Previously ALPS type Im.
ALPS-FASLG	*FASLG*	Germ-line mutations in *FASLG*. Previously ALPS type Ib.
ALPS-CASP10	*CASP10*	Germ-line mutations in *CASP10*. Previously ALPS type IIa.
ALPS-U	Unknown	Meets ALPS criteria but with undetermined genetic defect. Previously ALPS type III.
ALPS related disorders		
Revised nomenclature	**Gene**	**Definition**
CEDS: Caspase-8 deficiency state	*CASP8*	Germ-line mutations in *CASP8*. Lymphadenopathy and/or splenomegaly, marginal elevation of DNT cells, recurrent infections. Previously ALPS type IIb.
RALD: Ras-associated autoimmune leukoproliferative disease	*NRAS*	Somatic mutations in *NRAS*. Autoimmunity, lymphadenopathy, and/or splenomegaly, normal to elevated DNT cells, defective in vitro Fas-mediated apoptosis. Previously ALPS type IV.
DALD: Dianzani autoimmune lymphoproliferative disease	Unknown	ALPS-like signs but with normal DNT cells and defective in vitro FAS-mediated apoptosis
XLP1: X-linked lymphoproliferative disease	*SH2D1A*	*SAP* Germ-line mutations in *SH2D1A* or *SAP*. Fulminant Epstein-Barr virus infections, hypogammaglobulinemia, or lymphoma. Defective apoptosis in response to TCR restimulation

0505

ROMIPLOSTIM INCREASES AND MAINTAINS PLATELET COUNTS IN ADULTS WITH NEWLY DIAGNOSED OR PERSISTENT PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) WHO HAVE NOT UNDERGONE SPLENECTOMY

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Background. Deferral of splenectomy is recommended for at least 6-12 months following diagnosis of adult ITP to allow for spontaneous remission (Provan, *Blood* 2010). Traditional ITP therapies which are used in the interim have efficacy and tolerability limitations, with some used off-label, e.g. rituximab, which renders vaccines to prevent post-splenectomy infection ineffective for up to 6 months. Romiplostim is recommended for second-line treatment of adult ITP. **Aims.** To investigate the use of romiplostim in adults with newly diagnosed or persistent ITP who have not undergone splenectomy. **Methods.** This retrospective, pooled analysis included non-splenectomised patients, ≥18 years old, diagnosed with ITP for ≤1 year, who had received romiplostim in a randomized, standard of care study (Kuter, *N Engl J Med* 2011), or a single-arm study of patients with ITP of varying duration and severity (Janssens, *ASH* 2011). The analysis was descriptive, included data from patients' first 52 weeks on study, and summarized the following: platelet response, romiplostim dose, serious adverse events (AEs), and bleeding events. As per the original studies, platelet response was defined as a platelet count ≥ 50 x 10^9/L, with no rescue medications in the preceding 8 weeks. The proportion of time during which patients achieved a platelet response was calculated as: number of weeks with response/number of weeks with non-missing platelet counts. **Results.** Of the 92 patients analysed, 55% (51/92) had completed 52 weeks of treatment, and 23% (21/92) were continuing in their respective study (with less than 52 weeks of treatment). Median (Q1, Q3) age was 59 (36.0, 72.0) years, median time since ITP diagnosis 2.94 (1.20, 6.60) months, and mean (SD) baseline platelet count 22.6 (14.1) x 10^9/L. Median (Q1, Q3) duration of exposure to romiplostim was 51.86 (14.43, 52.00) weeks. Platelet counts rose rapidly during the first 3 weeks of romiplostim treatment, with median platelet count consistently above 100 x 10^9/L thereafter. Overall, 88% (81/92) of patients achieved a platelet response. On average, these patients had a response during three-quarters of the 52-week time period analysed (median [Q1, Q3], 78.9% [47.1%, 94.4%]). Romiplostim dose over time was similar to that reported previously (Kuter, *Lancet* 2008). Taking the average weekly dose of all patients, the median (Q1, Q3) was 3.00 (2.00, 5.06) µg/kg/week. Serious AEs occurred in 20% (18/91) of patients, with thrombocytopenia the most common non-bleeding event (2 [2%] patients). Fewer than half (43/91 [47%]) of all patients experienced bleeding events. Serious bleeds were rare (5 [6%] patients), with rectal hemorrhage and epistaxis the most common events (2 [2%] patients each). Thromboembolic events occurred in 2 (2%) patients (pulmonary embolism and thrombophlebitis [right arm], 1 patient [1%] each). **Summary and Conclusions.** With similar doses as reported previously, and no new safety signals, romiplostim maintained increased platelet counts in patients with newly diagnosed or persistent ITP who had not undergone splenectomy. Romiplostim may be an important treatment option when splenectomy is deferred in such patients, potentially reducing the risk of serious bleeding in those subsequently proceeding to splenectomy without impacting vaccinations administered to prevent post-splenectomy infection.

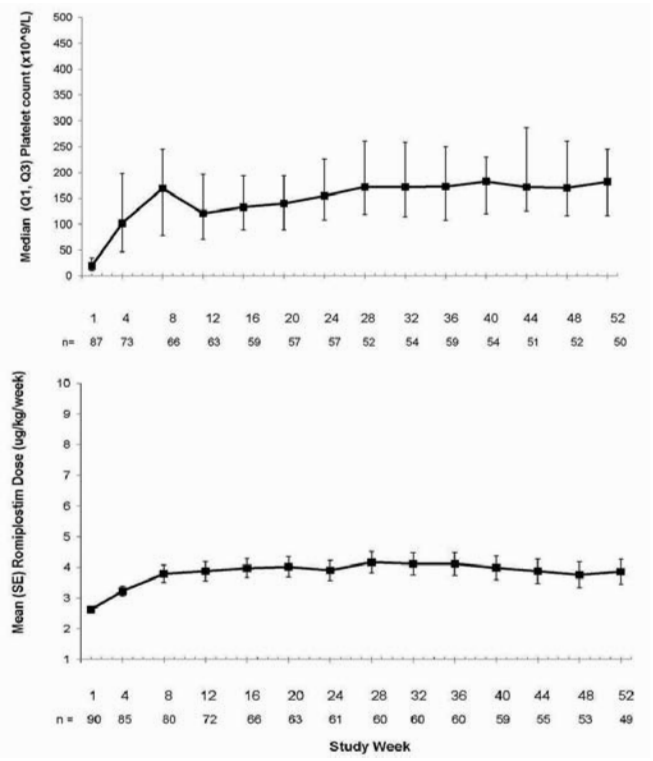

Figure 1. Platelet counts and romiplostim dose over time.

0506

BEYOND STANDARD PLATELET MEASURES, PREDICTORS OF BLEEDING SEVERITY IN IMMUNE THROMBOCYTOPENIC PURPURA

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Background. Immune thrombocytopenic purpura (ITP) comprises a heterogeneous group of disorders characterized by autoimmune mediated platelet destruction and impaired thrombopoiesis. Platelet count is frequently used to define bleeding risk with a platelet count of ≥30x 10^9/L generally considered haemostatic. Clinical bleeding heterogeneity exists in patients despite similar

platelet counts. This may provide false reassurance or, conversely, exposure to unnecessary treatment complications. A global assay, thromboelastometry, provides measures of haemostasis and fibrinolysis (Table 1). Measuring newly-formed platelets, immature platelet fraction (%IPF) and absolute immature platelet fraction (A-IPF), may also contribute insights to ITP bleeding severity. **Aims.** We hypothesized measures of thromboelastometry and IPF may better predict bleeding severity in ITP than platelet count and mean platelet volume (MPV). **Methods.** A prospective institutional-review-board-approved study enrolled 117 consenting patients with known ITP at a single center. Thromboelastometry whole blood clot formation profiles in patients were recorded by ROTEM® (Luddington, Clin Lab Haematol 2005). Four ROTEM assays were performed: INTEM and EXTEM representing initiation of intrinsic and extrinsic pathways respectively; APTEM to confirm possible hyperfibrinolysis; and FIBTEM measuring fibrin clot formation after blocking platelets. Blood counts were analyzed using Bayer-ADVIA 120 (Giacomini, Clin Lab Haemotol 2001). A-IPF and %IPF were determined by Sysmex XE2100 (Barsam, Blood 2011). Acute and chronic bleeding histories were evaluated per the ITP bleeding score (Page, BJ Haem 2007). **Results.** The platelet count, but not MPV, significantly correlated with acute bleeding score (ABS) (r=-0.32, P=0.0005). None of the FIBTEM parameters significantly correlated with ABS. INTEM, EXTEM and APTEM parameters correlated significantly (P=<0.0001) with ABS almost always demonstrating a greater correlation with ABS than observed with platelet count (Table 1). Mann-Whitney U subgroup analysis of subjects with platelet <60x10^9/L (n=53) compared subjects with ABS 0-2 (asymptomatic or ≤2 cutaneous symptoms) to those with ABS ≥3 and demonstrated that the INTEM, EXTEM and APTEM parameters (except CT) outlined in table 1 differed significantly (P≤0.0002). Among subjects with IPF data (n=86), significant correlations were observed with ABS versus %IPF (r=0.30, P=0.006) and ABS versus A-IPF (r=-0.30, P=0.006); however, ABS correlated more strongly with platelet count (r=-0.35, P=0.001). **Conclusions.** Measures of clot formation, clot firmness, and both clotting and fibrinolysis more strongly correlated with ABS than platelet count. Analyzing only subjects with platelets <60x10^9/L, the outlined (Table 1) significant assay parameters differed significantly among subjects with ABS 0-2 versus those with ABS ≥3. These findings suggest thromboelastometry may improve identification of patients at risk of bleeding and thereby guide management decisions as well or better than standard platelet counting. The same parameters being significant across INTEM, EXTEM and APTEM assays, but not FIBTEM, support the global role of platelets in haemostasis and suggest the need to define platelet function in ITP to appropriately predict bleeding. The lesser significance of %IPF and A-IPF with ABS suggests younger platelets may not be the dominant factor influencing bleeding. Further work will study additional patients at lower counts, explore these parameters in children with ITP, and better characterize factors underlying platelet function and bleeding in ITP patients.

Table 1. Correlation of acute bleeding score with ROTEM assay parameters.

		INTEM		EXTEM		APTEM		FIBTEM	
		r	P-value	*r*	P-value	*r*	P-value	*r*	P-value
Clot formation	CT	-	ns	-	-	-	ns	-	ns
	CFT	0.42*	<0.0001	0.39*	<0.0001	0.34*	<0.0001	-	ns
Clot Firmness	A10	-0.40*	<0.0001	-0.38*	<0.0001	-0.38*	<0.0001	-	ns
	A20	-0.40*	<0.0001	-0.39*	<0.0001	-0.39*	<0.0001	-	ns
	MCF	-0.39*	<0.0001	-0.38*	<0.0001	-0.38*	<0.0001	-	ns
	MCF-t	0.35*	<0.0001	0.39*	<0.0001	0.34*	<0.0001	-	ns
Clot Lysis	ML	-0.33*	0.0004	-0.26	0.006	-0.24	0.02	-	ns
	LI45	0.29	0.0016	0.34*	0.0002	0.29	0.001	-	ns
	LI60	0.28	0.003	0.30	0.001	-	ns	-	ns
Clot formation & lysis	AUC	-0.39*	<0.001	-0.38*	<0.0001	-0.34*	0.0002	-	ns

*Indicates parameters that demonstrated a stronger correlation with acute bleeding score than that observed with platelet count.

ns=not significant; CFT=clot formation time; A10 and A20=clot amplitude at 10 and 20 minutes respectively; MCF=maximal clot firmness; MCF-t time to maximal clot firmness; ML=maximal lysis; LI 45 and LI 60= lysis index at 45 and 60 minutes respectively; AUC=area under the curve

0507

ISOLATION AND EXPANSION OF DIFFERENT MEGAKARYOCYTE PROGENITORS AND THEIR SUBSETS IN NORMAL AND DISEASE STATES

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Background. Platelet production is regulated by physiological demand and entails several developmental steps beginning with hematopoietic stem cell (HSC) commitment to megakaryocyte progenitor progenitors (Mk-p), proliferation of Mk-p, terminal Mk maturation, and proplatelet production. While each step in this complex pathway is crucial for platelet production, the megakaryocyte progenitor pool provides the platform for platelet development. **Aims.** The aim of this study was to assess the quantity and properties of HSC and MK-p and their subpopulations in normal and myeloproliferative disease states **Methods.** Using high definition flow cytometry (HDFC) we resolved, characterized and isolated these populations. The recently described CD41^{high/ SSC low /CD45 dim/neg} Mk-p (1) were sorted and colony forming capacity tested. The populations studied were from normal bone marrow-BM, cord blood-CB, mobilized peripheral blood, and BM from Immune Thrombocytopenia Purpura. Results. We demonstrate for the first time that the relative proportion of early Mk-p including CD41^{high/ SSC low /CD45 dim/neg} that still retain CD34 and CD34+ HSPC that acquired high levels of CD41 vary under different physiological conditions. $CD34^+$ and $CD34^+/CD41^+$ cells were increased in PBSC and decreased in CB, correlating with the known shorter platelet nadir in patients transplanted with PBSC and the protracted thrombocytopenia following CB transplant. Further analysis of normal BM and PBSC resolved that 1-2% of the CD41^{high/ SSC low /CD45 dim/neg} MK-p remained early progenitors expressing CD34. The same MK-p population in CB contained no detectable CD34+ cells, once again pointing to reduced numbers of transplantable early Mk-p in CB. In CML and ET, where platelet counts are high $CD34^+/CD41^+$ cells were reduced, implying forced maturation of MK-p and facilitated thrombopoiesis. We further resolved the subpopulations of Mk-p that maintained CD34 in ITP, CML and ET and found a higher proportion of these early Mk-p in ITP and CML. In ET which is characterized by MK maturation and increase platelet production, the proportion of CD41^{high/ SSC low /CD45 dim/neg} Mk-p was increased 10X over normalBM, yet few CD34+ cells were noted, again implying accelerated maturation and loss of CD34. The CD41^{high/ SSC low /CD45 dim/neg} MK-p population from normal and disease states was sorted by HDFC and multiple gating and CFU-MK assays performed with the addition of thrombopoietin. The sorted cells have a uniform stem cell like morphology with a 5-10 fold enrichment in CFU-MK over the unsorted fraction. Sorted Mk-p from JAK mutations+ ET patients displayed a 10 fold increase in CFU-MK formation over all other samples tested. This increment was independent of TPO confirming the original FC analysis data. Sorted Mk-p were expanded 3 fold over one week in culture. Studying Mk-p under normal and disease states has now provided new information about these rare populations that are key contributors to platelet production and indicate that these progenitors may be appropriate for developing cellularexpansion strategies to treat thrombocytopenia. Further investigation of purified Mk-p may provide us with new insights into the molecular and cellular mechanisms that regulate normal and aberrant thrombopoiesis

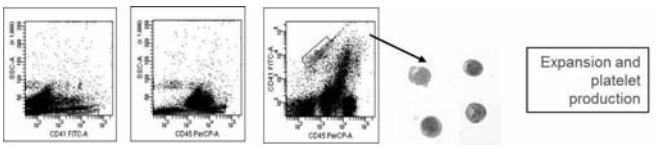

Figure 1. Gating and sorting of CD41high SSClow CD45 dim/neg Mk-P.

0508

INCREASE IN PLATELET COUNT AFTER FIRST DOSE OF ROMIPLOSTIM PREDICTS DIFFERENT DOSING REQUIREMENTS DURING THE FIRST SIX MONTHS OF TREATMENT IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

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Background. Patients treated with romiplostim for immune thrombocytopenic purpura (ITP) are more susceptible to variations in dosage during the first 6 months of treatment[1]. Splenectomized patients require higher dosages during this period[2]. Other predictors of different dosing requirements among patients throughout this period may contribute to easily achieve a stable dosage. **Aims.** To determine whether increase in platelet count after first dose of romiplostim is able to predict different dosing requirements among ITP patients during the first six months of treatment. **Methods.** 14 adult ITP patients with previous treatment failure started romiplostim in our Day Hospital between May 2010 and April 2011. Median age was 68 years (range 59-91), median weight was 77 kg (range 58-110). 5/14 (35,7%) of patients were splenectomized. Romiplostim was administered every 7±1 days starting from 1 mcg/kg. Dose was adjusted weekly on the basis of platelet response (PLT < 50 x 10^9/l: dose increased by 1 mcg/kg; PLT 50-400 x 10^9/l: dose maintained; PLT > 400 x 10^9/l: dose withheld and restarted reduced by 1 mcg/kg when PLT < 200 x 10^9/l). Concomitant steroid therapy remained unchanged until platelet count was ≥ 50 x 10^9/l, then was tapered up to termination. Weekly platelet counts and dosages were recorded during the first 24 weeks. Patients were divided in two groups on the basis of an increase in platelet count ≥ 30 x 10^9/l or smaller after first dose of romiplostim. Median dosages of romiplostim during the first 24 weeks were calculated and compared between the two groups using the two tailed Mann-Whitney U test at a significance level of 0,05. **Results.** 7 patients (2 splenectomized, 28,6%) had an increase in platelet count ≥ 30 x 10^9/l after first dose of romiplostim (median 83 x 10^9/l, range 12-414) and 7 (3 splenectomized, 42,9%) had a smaller one (median 11 x 10^9/l, range 1-100). Median weight was77 kg (range 58-56) and75 kg (range 58-110) respectively. Median dosage of romiplostim throughout the first 24 weeks of treatment was 2 mcg/kg (range 0-7) and 3 mcg/kg (range 0-10) respectively and the difference was statistically significant (p < 0,001). Figure 1 summarizes weekly median dosages of both groups. **Conclusions**. Patients achieving an increase in platelet count ≥ 30 x 10^9/l after first dose of romiplostim required a lower median dosage and had a more narrow range of dose during the first six months of treatment. However, an influence of the higher proportion of splenectomized patients in the group with a smaller increase cannot be excluded. Thus, in our experience increase in platelet count after first dose of romiplostim was predictive of different dosing requirements during the first six months of treatment but these data need to be confirmed in larger perspective studies

References

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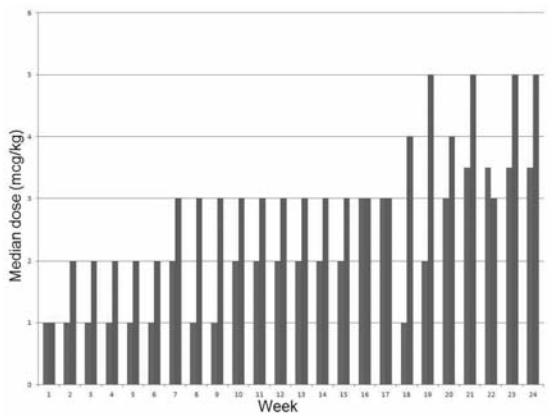

Figure 1. Weekly median dosages of romiplostim in patients with response to first dose ≥ 30 x 109/l platelets (blue columns) or lower (red columns).

0509

IMPAIRED PLATELET PHOSPHATIDYLSERINE EXTERNALIZATION AND CIRCULATING PLATELET-DERIVED MICROPARTICLES AFTER STRENUOUS EXERCISE IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT INDIVIDUALS

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Background. It was previously reported that glucose-6-phosphate dehydrogenase (G6PD)-deficient mice suffered from an increase in oxidative stress, as manifested by a decrease in NADPH and intracellular glutathione levels, and an increase in lipid peroxidation marker. These oxidation-induced changes in the membrane contribute to phosphatidylserine externalization, the accelerated senescence and random destruction of blood cells. **Aims.** This study aimed to determine whether decreased G6PD activity after a strenuous exercise can cause deleterious changes in membrane phospholipid asymmetry and an increase production of cell-derived microparticles (MPs). **Methods.** Eighteen individuals with G6PD Viangchan variance (871G>A) and matched healthy subjects were allowed to perform running on a treadmill at their maximal oxygen uptake. We quantified and identified the cellular origin of circulating MPs (red blood cells, platelets, and endothelial cells) in G6PD deficient individuals using flow cytometry. We also investigated the association between the level of MPs and several blood redox status markers including the total antioxidant capacity, the level of lipid peroxidation, the antioxidant enzyme activities, such as glutathione peroxidase (GPx), and superoxide dismutase, and G6PD. **Results.** The results showed that the concentration of platelet-derives MPs (PMPs) and the amount of the phosphatidylserine externalization on outer leaflet of platelets were decreased in G6PD deficient individuals after the strenuous exercise as compared to healthy subjects (P<0.05). After 45 min of recovery, the concentration of PMPs returned to basal values in both groups. The G6PD activity was decreased during exercise and continue to low in recovery period in G6PD deficient individuals (P<0.05), while healthy subjects had no change of G6PD activity during exercise. This study demonstrated that the amount of platelet-phosphatidylserine externalization was related to the activities of G6PD (r = -0.342, P<0.05) and GPx (r = 0.300, P<0.05), and the concentration of malondialdehyde - lipid peroxidation marker (r = 0.404, P<0.05). **Conclusions.** This study suggested that the amount of PMPs was increased in patients with Viangchan (871G>A) G6PD deficiency induced by an acute strenuous exercise and may correlate with the impairment of antioxidant status. The knowledge from this study may help the G6PD patient in prevention toward severe hemolysis inducing by strenuous exercise.

0510

THE ROLE OF DNA METHYLTRANSFERASE 3A MRNA EXPRESSION IN EGYPTIAN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. Idiopathic thrombocytopenic purpura (ITP) is an organ specific autoimmune hemorrhagic disease characterized by breakdown of self tolerance and triggering auto-reactive lymphocytes' response against platelets. The underlying etiology of ITP remains largely unknown. DNA methylation plays an essential role in maintaining T-cell function, and impaired methylation can lead to inappropriate gene expression and contribute to T-cell auto-reactivity and autoimmunity. **Aims.** This study aimed to evaluate the role of DNA methyltransferase 3A gene expression in the pathogenesis of ITP. **Methods.** This study included 60 subjects; 20 healthy volunteers as a control group, 20 patients with acute ITP and 20 patients with chronic ITP. DNA methyltransferase 3A (DNMT3A) mRNA expression in peripheral blood mononuclear cells was measured by real-time quantitative polymerase chain reaction. Plasma S-adenosylhomocysteine (SAH) levels were assayed with reversed-phase high performance liquid chromatography. All cases gave informed consent to participate in this study. **Results.** DNMT3A mRNA expression was significantly decreased in ITP patients as compared with the control group. Plasma SAH level was significantly elevated in ITP patients than in healthy controls. However, no significant difference was found in DNMT3A mRNA expression or plasma SAH level between acute and chronic ITP patients. **Summary and Conclusions.** Aberrant DNA methylation status reflected by decreased mRNA expression of DNMT3A and increased plasma SAH level may play an important role in the pathogenesis of ITP, although the precise underlying mechanisms still await further investigations and extensive work in this field is clearly needed to provide novel therapeutic targets for ITP.

0511

EFFICACY AND SAFETY OF SPLENECTOMY IN ADULTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA:SINGLE INSTITUTION STUDY

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Background. The spleen in immune thrombocytopenic purpura (ITP) patients is an important source of anti-platelet antibodies and the major site of platelet destruction. Splenectomy is the only treatment known to have curative effects in a susbstantial fraction of ITP patients. However in modern era of new thrombopoietic agents the place of splenectomy is somewhat uncertain. This study included patients with ITP who underwent splenectomy between 2004.-2009 at single medical center in Serbia. **Material and Methods.** We analyzed data of 116 patients (female 81, male 35) who underwent splenectomy for the treatment of ITP between December 2004 to January 2009 in Clinical Center of Serbia with a postoperative follow up between 16 and 64 months, median). The median age of the patients was 37 year (range 18-80), with median platelet counts before splenectomy 23x10^9/L (range 1-87x10^9/L). All patients before splenectomy were treated with prednison and 35 patients with other therapeutic procedures as: 18 patients with IVIg, 5 with danazol, 8 with azathioprin, 2 with vincristin loaded platelets and 2 with cyclophosphamide. In this cohort of 35 patients the number of platelets before splenectomy was below 20x10^9/L. All 18 patients who got IVIg at a doses of 0.400 mg/kg body weight for 5 days achieved a platelet counts >100 x10^9/L. All 116 patients received preoperative pneumococcal vaccination as well as steroid preparation. The spleens were removed with classical surgery and in 17 patients an accessory spleen was found and removed. The average weight of the spleen was 151 g (range 90-500g) and there was no major alterations in histology of the spleens. **Results.** After splenectomy 73 patients had a excellent platelet response over a mean follow up of 11 months (range 12/64). Their steroid therapy was tapered within the first week after operation. In 24 patients there were an increase of platelet count above 500 x109/l so they were under aspirin and 5 of them even under LMW heparin until the normalization of platelet count. Another 9 patients had partial response. Ten patients were considered nonresponders to primary splenectomy and continued to require immunosuppressive therapy to maintain the platelet count above dangerous level or at the time of bleeding even IVIg. There was no operative mortality. The operative morbidity was subphrenic abscesses 1(he required drainage) and 4 patients had a small collection of liquid in the place of removed spleen, 3 patients had postoperative bleeding, 2 patient were operated the same day and one patient the day after splenectomy, one patient (heavily and long treated with corticosteroids) had tuberculosis miliaris, one patient cardiac arrest (but recovered), one thrombosis of v.portae and gangrene of small intestine (she had normal platelet count and recovered after resection), one patient pancreatic fistula. All patients recovered completely. **Conclusions**. This study shows that splenectomy is a safe procedure and effective in approximately two thirds of patients with chronic ITP. This therapeutic procedure still remains first line treatment for the majority of patients with ITP.

0512

SUDDEN FLUCTUATIONS OF WEEKLY PLATELET COUNT (SFPC) ARE RARELY INDICATIVE OF CHANGES IN THE EFFECTIVENESS OF ROMIPLOSTIM IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

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Background. Romiplostim is an effective treatment for chronic immune thrombocytopenic purpura (ITP). Recently it has been pointed out that frequent changes in dose may create great fluctuations in platelet count, and therefore should be avoided whenever possible[1]. However, most of patients require up to six months before achieving a stable dosage[2]. Sudden Fluctuations of weekly Platelet Count (SFPC) within the range 50-400 x 10^9/l are common during this period leading sometimes to pre-emptive dose changes. **Aims** To determine whether SFPC are indicative of changes in the effectiveness of romiplostim and predict the necessity of changing dosage. **Methods.** 14 adult ITP patients with previous treatment failure started romiplostim in our Day Hospital between May 2010 and April 2011. Median age was 68 years (range 59-91), median weight was 77 kg (range 58-110). 5/14 (35,7%) of patients were splenectomized. Romiplostim was administered every 7±1 days starting from 1 mcg/kg. Dose was adjusted weekly on the basis of platelet response (PLT < 50x10^9/L: dose increased by 1 mcg/kg; PLT 50-400x10^9/L: dose maintained; PLT > 400x10^9/l: dose withheld and restarted reduced by 1 mcg/kg when PLT <200x10^9/L). Weekly platelet count was assessed in all patients during the first 24 weeks, for a total of 327 evaluable counts (9 counts were excluded because assessed in a different laboratory). SFPC were defined as increases or reductions ≥ 50x10^9/L platelets within the range 50-400x10^9/L without changes in two weeks previous neither in dose of romiplostim nor in concomitant therapy. SFPC were considered indicative of changes in the effectiveness of romiplostim if in 2 weeks a variation in dose of romiplostim congruous with the type of oscillation was needed (e.g.: for increases in platelets a congruous variation was a reduction in dose). **Results.** Incidence of SFPC on total counts was 11,6% (38/327). Among SFPC, 21,1% (8/38) was indicative of changes in effectiveness of romiplostim and 78,9% (30/38) was not. 92,8% (13/14) of patients had at least 1 not indicative SFPC (range events per patient: 0-4), while only 57,1% (8/14) experienced an indicative event (range events per patient: 0-1). Table 1 summarizes differences between indicative and not indicative SFPC. Incidence of indicative SFPC on total counts of splenectomized and not splenectomized patients was 2,5% (3/118) and 2,3% (5/209) respectively. **Conclusions** SFPC were common during the first six months of treatment, but only a minority of them was indicative of changes in effectiveness of romiplostim with similar incidence between splenectomized and not splenectomized patients. Besides, quite all the patients experienced one or more not indicative SFPC while indicative ones regarded few more than half patients and never twice the same subject. Therefore, pre-emptive changes of dose when SFPC occur should be avoided and an observation period of 1 week may be considered also for SFPC slightly out of range

References

1 Kuter DJ. *Cancer Treat Res*. 2011;157:267-288
2 Bussel JB, Kuter DJ, Pullarkat V, et al. *Blood.* 2009;113:2161-2171

Table 1 Differences between indicative and not indicative SFPC

	Indicative SFPC	Not indicative SFPC
Events on total SFPC (%)	8/38 (21,1)	30/38 (78,9)
Patients with at least 1 event on total patients (%)	8/14 (57,1)	13/14 (92,8)
Range events per patient	0-1	0-4

Drug resistance

0513

DUAL GSTT1 AND GSTM1 GENE DELETIONS INCREASE THE RISK OF IMATINIB FAILURE IN CHRONIC MYELOID LEUKAEMIA (CML)

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Background. GSTs are highly abundant phase II metabolising enzymes that are responsible for the conjugation of glutathione onto reactive drug intermediates. Deficiencies in enzyme activity may increase the risk of malignancies by allowing unconjugated intermediates to cause DNA damage. Alternatively, high enzyme activity may contribute to cancer drug resistance by increasing drug metabolism. The most commonly reported GST defects are whole gene deletions of GSTT1 and GSTM1, carried by 20% and 50% of the Caucasian population respectively.In CML, a meta-analysis of 9 studies combining 757 CML cases versus 1959 healthy controls of mixed ethnic groups associated deletions of GSTT1 with an increased risk of developing CML. A further recent study demonstrated that GSTT1del, either alone or with GSTM1del, increased the risk of CML. However, the effect of GSTT1 and GSTM1 gene deletions on imatinib response in CML has not been investigated. **Aims.** To study the effect that GSTM1 and/or GSTT1 deletions, or gene copy number, have on response to imatinib treatment in CML patients. **Methods.** 193 chronic-phase CML patients treated from original diagnosis with imatinib (400mg daily) were included, from Liverpool, Glasgow, London (Hammersmith) and Mannheim. Imatinib failure was defined as a change of treatment from imatinib due to either intolerance or disease resistance in line with ELN recommendations. Time to imatinib failure was assessed by Kaplan-Meier survival plots using the Mantel-Cox log-rank statistical test (SPSS® software). GSTM1 and GSTT1 gene copy number analysis was performed on the ABI Prism 7900HT Sequence Detection System using pre-tested Taqman® assays with RNase P as an internal calibrator (Applied Biosystems®). Data were collected using the SDS software and copy number was determined by the ΔCt method using the CopyCaller® software. **Results.** Gene copy number amplification was frequently observed with the GSTT1 gene (44% with 3-11 copies), whereas copy number reduction/deletion was common with GSTM1 (88% with 1 or no copies). GSTM1 copy number was unrelated to time to imatinib failure (p = 0.936), however a statistically significant copy number concentration-dependent trend was seen with GSTT1 (p = 0.015). However, once data was stratified according to absence or presence of each gene, regardless of copy number, those patients with both gene deletions were at greater risk of imatinib failure (p = 0.007) (Figure 1): in addition, this appeared more likely to occur within 2 years of treatment. **Summary and Conclusions.** CML patients carrying both GSTT1 and GSTM1 gene deletions are more likely to fail imatinib treatment. Screening patients for these gene deletions at diagnosis may be useful in guiding personalised TKI treatment, and merits prospective investigation.

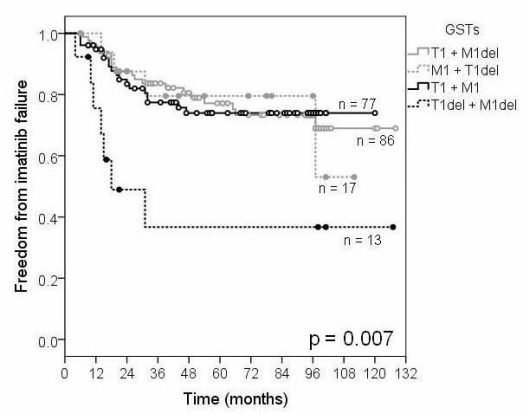

Figure 1. Kaplan-Meier log-rank survival curve of GSTT1 and GSTM1 gene deletions. Grey solid line/open grey circle = only the GSTT1 gene is present; grey dashe.

0514

TARGETING THE HEDGEHOG SIGNALING PATHWAY IN THERAPY-RESISTANT BCR-ABL1 POSITIVE LEUKEMIA

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An emerging concept in concept in cancer biology is that a rare population of cancer stem cells exists in the heterogeneous cell mass that constitutes a tumor. This concept also applies to BCR-ABL1 positive leukemia. Major advances also have been made in understanding the interactions between Hedgehog signaling and other pathways during carcinogenesis. Therefore, we examined the expression of Gli1 and BCR-ABL signaling pathways. Gli1 is regulated by BCR-ABL signaling, especially, mTOR/PI3K signaling pathways. Vismodegib is a selective Hedgehog pathway inhibitor that blocks hedgehog signaling by binding to Smo and inhibiting activation of downstream Hedgehog target genes. In the present study, we investigated the combined effects of vismodegib and ponatinib, a pan-ABL1 kinase inhibitor, in mutant forms of BCR-ABL1-expressing BaF3 cells and T315I-expressing human leukemia cell line, SK-9. To assess the *in vivo* efficacy of ponatinib and vismodegib, athymic nude mice were injected s.c. with BaF3 cells expressing wild-type (WT)-BCR-ABL1 and BCR-ABL1 mutants (M244V, G250E, Q252H, Y253F, E255K, T315A, T315I, F317L, F317V, M351T, H396P). 5 days after injection (average tumor volume, 100 mm^3), the mice were randomized into four groups (5 mice per group), with each group receiving either vehicle, ponatinib (30 mg/kg; q.d.), vismodegib (10 mg/kg; q.d.), ponatinib (30 mg/kg; q.d.) + vismodegib (10 mg/kg; q.d.). The ponatinib and vismodegib combination more effectively inhibited tumor growth in mice compared to either vehicle- or ponatinib- or vismodegib-treated mice. To investigate combined effects of vismodegib and ponatinib on T315I-expressing human leukemia cells, NOD/SCID mice were injected intravenously with SK-9 cells. Treatment with vismodegib and ponatinib demonstrated a marked segregation of apoptotic cells in both the central bone-marrow cavity, the endosteal surface, spleen and liver. The residual leukemia cells assayed by BCR-ABL mRNA were lowest in the vismodegib + ponatinib -treated mice, compared with the vehicle or ponatinib alone. To further investigate the effects of Hedgehog inhibition of self renewal and the relevance of the Hedgehog pathway as a therapeutic target in BCR-ABL1 positive leukemia, we examined the activity of vismodegib against SK-9 cells *in vivo*. NOD/SCID mice were injected with SK-9 cells then treated with vismodegib for 28 days. All mice demonstrated engaraftment of leukemia. We isolated human CD45+ cells from the spleen of mice from each treatment group and injected equivalent numbers of leukemia cells into secondary recipients, subsequently treated with vismodegib for 28 days. Following 60 days, all mice receiving SK-9 cells from vehicle treated mice engrafted with leukemia. In contrast, leukemia engraftment was detected in only 2/5 recipient mice receiving SK-9 cells from vismodegib treated donors. These results demonstrate persistent effects of Hedgehog inhibition on the long term self-renewing BCR-ABL1-positive leukemia cells. These results suggest that the combination with a Smo inhibitor and ABL1 tyrosine kinase inhibitors (TKIs) may help to eliminate the therapy-resistant T315I BCR-ABL1 positive leukemia cells.

0515

PREDICTION OF CYTARABINE CYTOTOXICITY IN ACUTE MYELOID LEUKAEMIA PATIENT SAMPLES USING A RAPID IN-VITRO BIOSENSOR ASSAY: CORRELATION WITH CLINICAL OUTCOMES

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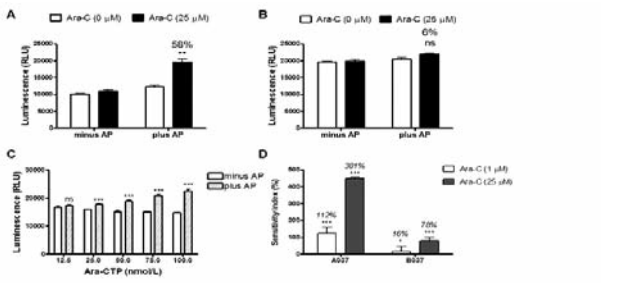
Background. Acute Myeloid Leukaemia (AML) is treated routinely (except for Acute Promyelocytic Leukaemia) with Cytarabine (Ara-C) at varying dose (20 mg/M^2/d to 3G/M^2/d) in combination with other cytotoxics. Regrettably, this fails to achieve CR in approximately 40% of cases, with resistance to chemotherapy, including cytarabine, being a major reason for failure. **Aims.** To correlate biosensor assay findings of Ara-C sensitivity of mononuclear cells from presentation blood/bone marrow of AML patients with clinical outcomes (CR or NR) after first course of cytarabine containing induction chemotherapy. **Methods.** We have previously validated a 8 hour *in vitro* bioluminescent bacterial biosensor assay which measures Ara-CTP (active metabolite of Ara-C) generation inside AML cells exposed to 1microM and 25 μM Ara-C (equivalent to 20 mg/M^2

and 200 mg/M2 respectively) for 30 minutes, in leukemic cell lines (Alloush *et al*. Clin. Chem. 56: 1862-70, 2010). Briefly, after AML cell incubation with Ara-C, Ara-CTP levels in cell lysates are deduced by increase in light generated by the reporter MG1655 *E.coli* strain (which has been genetically modified to permit this function: Dcdd +dCK +lux). Bioluminescent light emission is compared with internal controls and any increase above 13% is deemed a positive result indicating AML cell capability to produce Ara-CTP and hence Ara-C sensitivity. Biosensor data was validated against results obtained from a 3 day commercial cytotox assay, flow cytometric viability studies and HPLC assessment of Ara-CTP production (data not shown). This paper extends our experience of this assay using diagnostic blood/bone marrow samples in 71 AML patients and for the first time attempts to correlate the assay with known clinical outcome in 53 of these patients. Examples of assay findings in patient samples with low and standard dose Ara-C are described in Figure 1. **Results.** In the 53 patients with known outcome (median age 64.5 years) the assay correctly predicted outcome in 44 (22 CR and 22 NR). The sensitivity range (SI%) for CR was 14 to 128% (median 36%) and for NR -9 to 7% (median 3.5%). Typical profiles are shown for a clinically responsive (Figure 1A) and non-responsive individuals (Figure 1B). ROC curve analysis again determined a cut-off sensitivity index of 13% (AUC=0.81, p<0.0001) which produced 5 false positives and 4 false negatives for the 53 patients. Sample quantity limited the investigation into the source of this error. Testing for response to Ara-C in the 53 presenting patient samples gave 83% efficiency (sensitivity 85%, specificity 82%) when correlated with remission status following actual treatment with Ara-C containing induction chemotherapy. **Conclusions.** By predicting chemosensitivity to varying doses of Ara-C, this assay could potentially offer a tailored treatment approach in AML. Selection of an optimal dose of cytarabine upfront could reduce the risk of induction failures and may also help prevent overtreatment of elderly patients whose AML is sensitive to lower doses of Ara-C. Prospective clinical correlation in a larger cohort of AML patients in the context of national clinical trials is planned with funding from the Medical Research Council in the UK.



Patient profiles using the biosensor assay (A and B), the limit of detection of the biosensor for Ara-CTP(C) and in vitro low dose (1 µM) and standard dose (25 µM) Ara-C testing in AML patients (D).
Effect of 30-minutetreatment with Ara-C (25 µM) on cells from a clinically Ara-C sensitive patient (A) and a clinically Ara-C resistant patient (B) analysed using the Ara-C biosensor assay.
(C) Peak light emission from the biosensor treated with Ara-CTP at 0 to 100 nM (n=3, error bars show range). Correlation between Ara-CTP and light emission ±AP produced an R2=0.9953.1 Reproducibility studies using calibrators with defined Ara-CTP levels have shown CV<10% (n=16) and using cell lines CV<5% (n=9).
(D) Cells from patients considered unfit for intensive chemotherapy were treated with in vivo equivalents to low and standard dose Ara-C (patient ages: A037=78 years and B037=63 years). The elderly patient (A037) shows a high SI% and could benefit from low dose Ara-C treatment, determinable due to the wide detection range of the biosensor (0.025 to 100 µM). Error bars represent standard deviation (n=3), statistical significance relative to control (*p<0.05, **p<0.01, ***p<0.001).

Figure 1.

0516

SMAC MIMETIC OVERCOMES APOPTOSIS RESISTANCE OF FADD- OR CASPASE-8-DEFICIENT ACUTE LYMPHOBLASTIC LEUKEMIA CELLS BY TRIGGERING NECROPTOSIS TOGETHER WITH TNF?

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Evasion of apoptosis contributes to treatment resistance, one of the major, yet unresolved obstacles in oncology. Searching for new strategies to bypass apoptosis resistance we investigated the potential of Smac mimetic in acute lymphoblastic leukemia (ALL) cells, which are deficient in FADD or caspase-8, two key signaling molecules in the extrinisic apoptosis pathway. Smac mimetic antagonizes Inhibitor of Apoptosis (IAP) proteins that are expressed at high levels in ALL. Here, we demonstrate for the first time that Smac mimetic primes apoptosis-resistant leukemia cells for TNFα-induced necroptosis as an alternative mode of cell death. The interaction of Smac mimetic and TNFα is highly synergistic as calculated by combination index (CI <0.1). Kinetic analysis reveals that Smac mimetic and TNFα cause cell death rapidly within few hours. Interestingly, Smac mimetic- and TNFα-mediated cell death in FADD-deficient cells occurs without characteristic features of apoptosis, namely without caspase activation or DNA fragmentation, pointing to non-apoptotic cell death. In sharp contrast, Smac mimetic and TNFα trigger activation of caspase-8, -9 and -3 and DNA fragmentation in apoptosis-proficient wildtype cells that express FADD and caspase-8. Consistently, the caspase inhibitor zVAD.fmk protects wildtype cells against Smac mimetic- and TNFα-triggered cell death, whereas zVAD.fmk fails to block cell death in FADD-deficient cells. Furthermore, production of reactive oxygen species (ROS) precedes Smac mimetic- and TNFα-induced cell death in FADD-deficient cells. In line with the notion that ROS contributes to cell death induction, the addition of ROS scavengers (i.e. butylated hydroxyanisole (BHA) and trolox) or the addition of the NADPH oxidase inhibitor diphenyliodonium (DPI) significantly reduce cell death upon combination treatment. Moreover, Smac mimetic- and TNFα-triggered cell death is accompanied by enhanced RIP1 kinase activity in FADD-deficient cells. Of note, Smac mimetic and TNFα cooperate to trigger the formation of the necrosome complex. Necrostatin-1, a RIP1 kinase inhibitor, reduces Smac mimetic- and TNFα-induced ROS generation and completely abolishes cell death in FADD-deficient cells, in accordance with necroptotic cell death. Importantly, Smac mimetic also sensitizes apoptosis-resistant, primary leukemic blasts derived from patients with ALL to TNFα-induced necroptosis, underscoring the clinical relevance of these findings.In conclusion, Smac mimetic primes leukemia cells to TNFα-induced apoptosis or necroptosis in a context-dependent manner. In apoptosis-proficient cells which express both FADD and caspase-8, Smac mimetic enhances TNFα-induced, caspase-dependent apoptosis. However in apoptosis-resistant cells lacking either FADD or caspase-8, Smac mimetic sensitizes ALL cells for TNFα-initiated necroptosis that critically depends on RIP1 and ROS production. These findings have important implications for the therapeutic exploitation of necroptosis as an alternative cell death program to overcome apoptosis resistance of cancers.

0517

FOLYLPOLYGLUTAMATE SYNTHETASE SPLICING ALTERATIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: IMPLICATIONS FOR MTX-THERAPY EFFICACY

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Background. Methotrexate (MTX) is one of the key antifolate drugs, commonly used in treatment of acute lymphoblastic leukemia (ALL) as well as other malignancies. In order for MTX to exert its full pharmacological activity, it must first undergo a unique intracellular metabolism known as polyglutamylation, catalyzed by the enzyme folylpoly-g-glutamate synthetase (FPGS). FPGS-dependent polyglutamylation of MTX results in intracellular retention and thus enhanced cytotoxicity. Hence, loss of FPGS function results in lower intracellular levels of MTX and as a consequence drug resistance. The mechanism underlying this phenomenon however remains elusive. Recent studies suggest FPGS mRNA splicing alterations as a possible contributor to MTX resistance (Stark et al, Blood 2009). Analysis of FPGS transcripts in specimens from adult ALL patients revealed exon 12 skipping, both at diagnosis and at relapse after high dose MTX-containing chemotherapy. Until now there are no reports available on this phenomenon in childhood ALL, therefore pediatric studies are warranted. **Aims.** The aim of the study is to evaluate FPGS mRNA splicing alteration patterns as a contributing factor to MTX treatment failure in childhood ALL patients. **Methods.** A PCR-based assay was devised that allows accurate screening of the entire FPGS mRNA sequence for aberrations in splicing. This method was subsequently used to screen childhood ALL patient samples and selected MTX-sensitive and MTX-resistant human leukemic cell lines. The identity of the most frequently appearing alterations was confirmed by sequencing. **Results.** The initial scan performed on a set of 14 childhood ALL patient specimens showed 7 different aberrations in FPGS mRNA splicing, including both exon skipping and intron retention. The detected alterations appeared with variable frequencies (ranging from 50% - 100%) among the samples. Most of these aberrations were either absent in specimens obtained from healthy individuals or present only at a very low abundance (Figure 1.). In addition, some of the alterations were found at an elevated level in several antifolate resistant cell lines (including MTX-resistant cells showing marked loss of FPGS activity with no substantial alterations in mRNA level) in comparison to their parental MTX-sensitive cell lines with proficient FPGS activity. **Conclusions.** Aberrancies in FPGS mRNA splicing seem to be a frequently occurring phenomenon in childhood ALL and may potentially be responsible for loss of FPGS activity. To which extent the identified FPGS splicing alterations contribute to MTX resistance and MTX treatment failure in childhood ALL in a clinical setting needs further investigation. Together these findings may assist in designing accurate and targeted tools that will be able to identify antifolate resistance

of leukemic cells. Ultimately these results may guide pediatric oncologists in efficacy assessments of MTX-containing anticancer treatment in individual patients (i.e. personalized medicine).

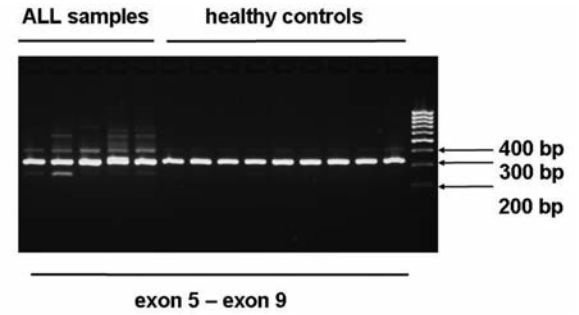

Figure 1. FPGS mRNA splicing alterations in childhood ALL samples in comparison to healthy individuals.

0518

CHARACTERISATION OF MINOR CML BLAST SUBSET THAT ARE SIGNIFICANTLY RESISTANT TO BOTH GROWTH INHIBITION AND APOPTOSIS MEDIATED BY IMATINIB AND NILOTINIB IN VITRO

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Background. Although very effective in chronic phase CML, imatinib (IM) usually less effective in advanced CML since drug-resistant clones inevitably shortly emerge. While nilotinib (NL) is more effective than IM, appearance of drug resistance to NL might be also relevant in advanced CML. We have previously found that in blast crisis CML, blasts of the same CML clone are heterogeneous, containing a small subset (1-3%) of blasts that are significantly more aggressive than the major malignant population (Differentiation. 76: 908-922, 2008). These minor subsets (MS) of blasts (both from patients and human, drug naive CML-BC cell lines) display higher clonogenicity than the common subsets (CS) and preferentially overexpress seven genes including BCR-ABL, transcription factors and extracellular matrix adhesion molecules. **Aims.** To evaluate whether the MS blasts also exhibit differential sensitivity toward IM and NL, we compared the two blast subsets for the effects of IM and NL on both proliferation and apoptosis. **Methods.** Various CS and MS blasts of CML-BC were evaluated before and after short exposure (24-48 h) to IM or NL *in vitro*. The anti-proliferative effects of IM and NL at various concentrations were measured by MTT proliferation assay. The apoptotic effects of IM and NL at various concentrations were measured by annexin-V assay and caspase-3 assay. **Results**. In the absence of drugs neither significant differences in the proliferation and apoptosis rates could be detected between the CS and the MS blasts. While IM (50-500 nM) and NL (10-50 nM) efficiently inhibited the proliferation of the CS blasts in a dose-dependent manner, the proliferation rate of the MS blasts was essentially not affected in all the tested concentrations. Similarly, IM at 500 nM and NL at 50 nM induced apoptosis of 60% and 90% of the CS blasts, respectively. However, the MS blasts were highly resistant to the apoptotic effects of IM and NL (Figure1). **Conclusions.** The existence of a minor 'pool' of CML blasts of both greater clonogenicity and aggressiveness, apparently signify clonal evolution toward both increased tumoreginicity and lower therapeutic sensitivity to both IM and NL. Specifically this study indicates that MS blasts are highly resistant to both the anti-proliferative and apoptotic effects of IM and NL. Future novel therapies should try targeting this minor aggressive blast cell population most probably by drug combinations, in order to improve outcome in advance CML.

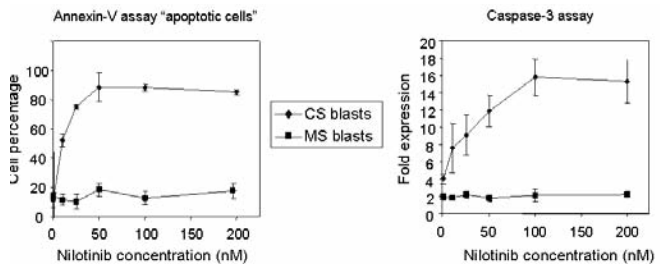

Figure 1. Apoptosis rate of MS versus CS blasts in the presence of nilotinib (NL) at various concentrations.

0519

THE HSP90-INHIBITOR AUY922 POTENTLY INHIBITS THE MOST FREQUENT BCR-ABL KINASE MUTATIONS EXCEPT T315I IN CHRONIC MYELOID LEUKEMIA CELLS

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Background. BCR-ABL kinase mutations are the most frequent reason for primary as well as secondary imatinib resistance in chronic myeloid leukemia. Recently differential antiproliferative activity against BCR-ABL mutations has been reported for the HSP90-inhibitor AUY922. Thus we asked whether AUY922 alone or in combination with imatinib would be able to overcome BCR-ABL dependent resistance. Recently Tauchi et al. showed a prevention of BCR-ABL mutation emergence when non-mutant BCR-ABL transformed cells were incubated with AUY922. Furthermore they demonstrated a significant inhibition of complete imatinib resistant T315I-BCR-ABL expressing cells. However we generated some controversial data. **Aims.** In vitro experiments were performed in order to characterize the activity of AUY922 alone and in combination with imatinib in BCR-ABL transformed cells. We further sought to analyze the potency of AUY922 to prevent the emergence of BCR-ABL kinase mutations. **Methods.** The murine hematopoietic cell line Ba/F3 and its derivative Ba/F3p210, as well as their mutant BCR-ABL expressing derivatives (Ba/F3p210^{M351T}, Ba/F3p210^{E255K}, Ba/F3p210^{Y253F}, Ba/F3p210^{T315I}) were used for MTS-based proliferation assays. Synergistic effects were calculated using a mathematical approach. Dose response experiments and subsequent Western blot analyses were performed to semiquantitatively determine BCR-ABL activity using pTyr as well as BCR-ABL specific antibodies. An N-Ethyl-N-Nitrosourea-(ENU)-based mutation screen was performed in non-mutant BCR-ABL transformed cells exposed to escalating doses of imatinib (0-16µM) +/- AUY922 (doses: 0.625 and 1.25nM ensured not to be antiproliferatively active). Primary cells of imatinib naïve patients as well as those of healthy donors were exposed to AUY922 (2.5 and 5nM) alone and in combination with imatinib (0.5 and 1 µM) to reveal differential antileukemic activity in colony forming assays. **Results.** Treatment of mutant BCR-ABL expressing cells using AUY922 as single agent revealed antileukemic activity in the nanomolar dose range. Although no significant difference was observed when IC50 values of mutant and non-mutant BCR-ABL expressing cells were compared. A synergistic activity was observed for both, mutant (Ba/F3p210^{M351T}, Ba/F3p210^{E255K}, Ba/F3p210^{Y253F}) and non-mutant (Ba/F3p210) BCR-ABL expressing cells, when AUY922 was combined with imatinib. Colony forming assays revealed the synergistic activity of AUY922 and imatinib for non-mutant BCR-ABL expressing primary cells.Furthermore protein analysis in non-mutant BCR-ABL transformed cells (Ba/F3p210) showed for AUY922 in combination with imatinib even at low doses (2.5nM) a significant BCR-ABL suppressive effect after 24 hrs. In contrast, T315I mutant cells (Ba/F3p210^{T315I}) on combined imatinib/AUY922 treatment were associated with unaffected cell growth and T315I-BCR-ABL protein expression levels.The hypothesis that addition of AUY922 might prevent imatinib treated BCR-ABL positive cells (Ba/F3p210) from emergence of BCR-ABL mutations was disproved in ENU-based mutation screens. In most cultures BCR-ABL kinase mutations were detected. Interestingly the occurrence of BCR-ABL mutation T315I was slightly enhanced (p=ns) in the presence of AUY922. **Summary and Conclusions.** In conclusion, our data provides further experimental evidence that AUY922 is a promising candidate to optimize ABL-inhibitor based CML therapy. AUY922 in combination with imatinib overcomes BCR-ABL dependent resistance in most frequent BCR-ABL mutations except T315I.

0520

POSSIBLE ROLE OF UGT2B17 GENE IN HAEMATOPOIETIC STEM CELL TRANSPLANTATION AS PHARMACOGENE

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In the hematopoietic stem cell transplant (HSCT) setting, the UDP-Glucuronil-trasferase 2B17, UGT2B17, is considered as a minor histocompatibility antigens (mHAg). Functionally, it belongs to a group of enzymes that catalyze the coniugation of glucuronic acid to a variety of substrates including steroid hormones, carcinogens, pollutants and chemotherapeutic drugs. Glucuronidation is implicated in the liver detoxification from noxious endogenous and exogenous compounds. The homozygous UGT2B17 gene deletion exists, is compatible with life, and is not rare, representing about the 15% in our population. People lacking this enzyme are constitutively poor metabolizers of steroid drugs and we questioned if this condition could represent an intrinsic risk of HSCT failure, for

a recipient, due to the inefficient metabolism of immune-suppression treatment and liver accumulation of toxic catabolites. To this, we analyzed retrospectively 156 Italian patients, 74 pediatric and 82 adult, 105 transplanted with an HLA-identical related donor and 51 unrelated. The frequency of the gene deletion, defined by PCR-SSP molecular technique, was 13.5% in the recipients and 14.1% in their donors. Ten years of follow up revealed that this condition associated significantly with death and relapse. In fact, 12/21 (57.14%) homozygous deleted patients died vs 38/135 (28.14%) (OR=3.409, p=0.008) and 11/16 (68.75%) relapsed vs 30/102 (29.4%) (OR=5.28, p=0.002) (see Kaplan-Meier curves in the Figure below). Pepe and Mori statistical test revealed that the constitutive absence of UGT2B17 influenced the relapse much more than the overall survival (p=0.0039). We also observed that, among the UGT2B17 homozygous deleted patients, the death rate was significantly higher in males (72.7%) than in females (40%) and in female to male couples, compared to all the other gender combinations (p=0.0013).In summary, we might suppose that the recipient's lack of UGT2B17 enzyme could be itself a risk factor for relapse and death independently from the mismatch. Beyond its documented role as a mHAg, UGT2B17 seems to have an important function in HSCT as a pharmacogene. The deleterious effect of UGT2B17 gene deletion seems to be even more prominent in deleted males receiving a transplant from a female donor. Our findings suggest that the UGTB217 molecular definition might be appropriate to identify the patients prone to the relapse and that sex matching should be preferred in UGT2B17 deleted male patients.

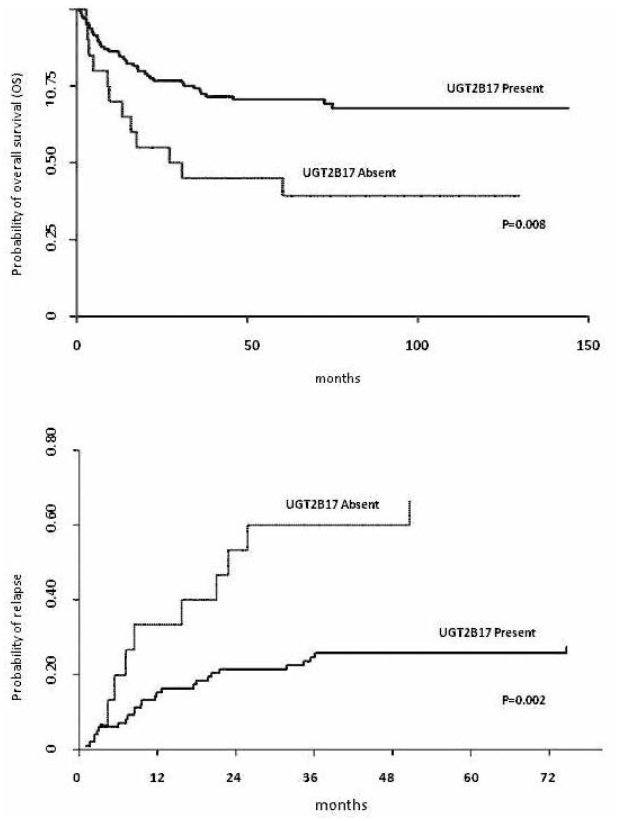

Figure 1. Influence of UGT2B17 deletion on overall survival and relapse.

0521

SUCCESSFUL CO-TREATMENT OF BCR-ABL LEUKEMIC CELLS WITH PONATINIB, AN ABL KINASE INHIBITOR, AND VORINOSTAT, A HISTONE DEACETYLASE INHIBITOR: A POTENTIAL TREATMENT FOR BCR-ABL POSITIVE LEUKEMIA CELLS

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Background. Resistance to imatinib is most commonly explained by acquired point mutations in the kinase domain of BCR-ABL, which impair drug binding. Ponatinib, also known as AP24534, is an oral, the multi-targeted tyrosine kinase inhibitor (TKI) and highly activity against ABL kinase point mutation included T315I. Ponatinib is currently being investigated in a pivotal phase 2 clinical trial (PACE trial). However, ABL1 inhibitor-resistant patients already harboring mutations have a higher likelihood of developing further mutations under the selective pressure of ABL1 TKIs. The challenge for development of an effective Philadelphia chromosome (Ph) positive leukemia therapy is therefore to develop an alternative treatment strategy that does not rely solely on kinase domain inhibition but rather results in degradation of the offending BCR-ABL1 protein regardless of its mutation status. Histone acetyltransferases (HAT) and histone deacetylases (HDAC) control the acetylation of histones and intracellular proteins, and regulate the transcription and function of the proteins. **Aims.** Combination therapy using a ponatinib and an HDAC inhibitor, vorinostat may help prevent development of Abl TKI resistance and may improve their long-term outcome. **Methods.** We analyzed the ponatininb and vorinostat efficacy by using the BCR-ABL positive cell line, K562 and murine Ba/F3 cell line which was transfected with imatinib resistant BCR-ABL random mutagenesis and T315I and E255K mutant cells and BCR-ABL positive primary samples. **Results.** 72 hours treatment of ponatinib exhibits cell growth inhibition and induced apoptosis against K562 cells. We also found that ponatinib potently induced cell growth inhibition of Ba/F3 T315I mutant cells. We found that phosphorylation of BCR-ABL, Crk-L was decreased and poly (ADP-ribose) polymerase (PARP) was activated. We also analyzed by mouse xenograft model. Combined treatment of Ba/F3 T315I mutant cells with vorinostat and ponatinib caused significantly more cytotoxicity than each drug alone. Although phosphorylation of BCR-ABL, Crk-L was not reduced after vorinostat treatment, acetylation of histone H4 was increased and BCR-ABL degradation was induced in the point mutant cells. Protein degradation of BCR-ABL was inhibited by co-treatment with lactacystin. Caspase 3 and PARP activation were increased after combination of ponatinib and vorinostat. Moreover, an increase in phosphorylation levels of γ-H2A.X was observed suggests that combination of ponatinib and vorinostat induced DNA damage against T315I mutant cells. We next established ponatinib resistant cells by using Ba/F3 BCR-ABL with resistant random mutants. In the ponatinib resistant cell lines, IC50 of ponatinib was 200 nM. BCR-ABL triple point mutations (T315I, E255K and Y253H) were detected by direct sequence and invader analysis. Ponatinib resistant Ba/F3 cells were also resistant to imatinib (IC50: more than 10μM) or nilotinib (IC50: 7.5 μM) and also resistant to dasatinib (IC50: more than 100nM). We investigated the efficacy between ponatinib and vorinostat by using these cell lines. Combined treatment of Ba/F3 ponatinib resistant cells with ponatinib and vorinostat caused significantly more cytotoxicity. **Summary**. Data from this study suggested that administration of the ponatinib and HDAC inhibitor, vorinostat may be a powerful strategy against BCR-ABL mutant cells and enhance cytotoxic effects of ponatinib in those BCR-ABL mutant cells.

0522

IMATINIB, BUT NOT NILOTINIB, IS ACTIVELY TRANSPORTED BY SLCO1A2 IN CHRONIC MYELOID LEUKAEMIA CELLS

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Background. We showed in 2004 that imatinib is actively transported into chronic myeloid leukaemia (CML) cells by human organic cation transporter 1 (hOCT1; SLC22A1). We and others have subsequently shown that a) CML patients with high levels/functional activity of hOCT1 have superior responses to imatinib; and b) hOCT1 does not transport the second generation tyrosine kinase inhibitors (2G TKI) dasatinib and nilotinib. However, there are many patients with low hOCT1 activity who respond well to imatinib. This suggests that other transporters may also transport imatinib and maybe 2G TKI.Hu et al showed that transfection of either SLCO1A2 (OATP1A2), SLC22A4 (OCTN1) or SLC22A5 (OCTN2) into *Xenopus* oocytes (which do not express any mammalian transporters) increased imatinib uptake, suggesting these transporters may be relevant to TKI transport in Man. **Aims.** To assess the role of SLCO1A2, SLC22A4 and SLC22A5 in TKI transport in CML cells and on treatment outcome. **Methods.** For transfection work, the CML cell line KCL22 was selected since it has low basal levels of transporter expression. KCL22 cells were transfected with pcDNA3-SLCO1A2 (kind gift of Prof M Fromm, Germany), the pcDNA-SLC22A4 and pcDNA-SLC22A5 plasmids (kind gift of Profs A Tsuji and Y Kato, Japan) and stable lines with high expression were selected. The time course of uptake of 5 μM radiolabelled imatinib or nilotinib into each of these lines was assessed, with and without the inhibitors naringin (for SLCO1A2 transfected) and verapamil (for SLC22A4 and A5 transfected) at 100 μM. Sixty-six newly diagnosed chronic phase CML cases receiving either imatinib (60) or nilotinib (6) as first line treatment were assessed for mRNA expression of SLCO1A2, SLC22A4 and SLC22A5 using TaqMan gene expression assays. Relative expression levels were normalised to GAPDH and compared to four normal controls. **Results.** Functional assays showed that uptake of imatinib into SLCO1A2 high expressing cells was significantly greater than into control cells ($p = 0.001$), and that this excess was blocked by the SLCO1A2 inhibitor naringin ($p=0.035$). In sharp contrast, uptake of nilotinib by SLCO1A2 high expressing cells did not differ from control cells. Neither imatinib nor nilotinib showed any enhanced uptake into CML cells over-expressing SLC22A4 or SLC22A5. There was no significant difference in diagnostic mRNA expression levels of SLCO1A2 between responders (defined as achieving complete cytogenetic remission (CCR) after 12 months treatment), non-responders (no CCR by 18 months) or patients who subsequently progressed to blast crisis. mRNA expression levels for SLC22A4 and SLC22A5 also showed no correlation to outcome. **Conclusions.** *In vitro*, imatinib is a substrate for SLCO1A2 but not for SLC22A4 and SLC22A5. The clinical significance of this is not clear and requires further assessment. Functional studies in clinical samples for patients with high expressing SLCO1A2 are in progress. Nilotinib is not transported by SLCO1A2, SLC22A4 or A5, and to date there is no evidence that it is transported actively into CML cells.

0523

POLYMORPHISMS IN ABC TRANSPORTER GENES ARE ASSOCIATED WITH COMPLETE MOLECULAR RESPONSE TO IMATINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. The resistance to the imatinib mesylate (IM) is the main cause of failure in the treatment of patients with chronic myeloid leukemia (CML). The efflux and influx membrane transporters are crucial elements in IM pharmacokinetics. Single nucleotides polymorphisms (SNPs) in membrane transporter genes could be related to resistance of this drug. **Aims.** To investigate the relationship between *ABCG2* (rs2231142 and rs1564481)*, SLCO1A2* (rs11568563), *SLCO1B3* (rs4149117, rs7311358 and rs17674290) and *ABCA3* (rs323043 and rs150929) SNPs and IM response in CML patients. **Methods.** One hundred and eighteen patients in the chronic phase of CML, both genders with an age range of 18 to 80 were studied. All patients were initially treated with a standard dose of IM (400 mg/day) and divided into two groups according to their response. The responder group comprised 70 patients who had a complete cytogenetic response within 18 months of treatment. The non-responder group comprised 48 patients who did not have a complete cytogenetic response with the initial dose (400 mg/day) of IM or who relapsed during treatment and were submitted to higher doses of 600 or 800 mg/day. Criteria of failed response to treatment were established by European LeukemiaNet. Patients with cytogenetic patterns other than the Philadelphia chromosome and/or with mutations in the BCR-ABL1 gene were excluded from this study. Major molecular response (MMR) was defined as a reduction of BCR-ABL1 transcript levels to ≤ 0.1% in the peripheral blood standardized on the International scale. Complete molecular response (CMR) was defined as a reduction ≤ 0.0032% BCR-ABL1 transcript levels. DNA from one hundred and twenty blood donors was also evaluated. The genotyping for ABCG2, SLCO1A2, SLCO1B3 and ABCA3 SNPs was performed by Real Time PCR. **Results.** None of the studied SNPs was related with increased risk of having CML. The distribution of genotypes for *ABCG2*, *SLCO1A2* and *ABCA3* SNPs was similar between responder and non-responder groups. There was a trend of higher frequency of GG (*SLCO1B3* rs7311358, p=0.055) and TT genotypes (*SLCO1B3* rs4149117, p=0.055) carriers in responder group. High frequency of *ABCA3* rs150929 GG+GT (N=51) genotype was found in group of poor CMR when compared with TT carriers (N=5, p= 0.014) in responder group. There was high frequency of CC genotype for ABCG2 rs2231142 in patients with CMR (p= 0.025). Furthermore, carriers of haplotype CC/GG (*ABCG2* rs2231142/rs1564481) had higher probability to achieve the CMR compared with the non-carriers (OR: 5.29; 95%CI: 1.06-26.28, p=0.042). None of the studied SNPs was associated with MMR. **Conclusions.** The haplotype CC/GG (*ABCG2* rs2231142/rs1564481) is associated with CMR, while *ABCA3* rs150929 GG+GT genotypes are related with poor CMR.

0524

DIMINISHED ASPARAGINASE CLEARANCE DUE TO A GERMLINE MUTATION IN CATHEPSIN B

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Background. Asparaginase (ASNase) is a key component of multi-agent chemotherapy protocols used in the treatment of pediatric as well as adult acute lymphoblastic leukemia (ALL). Patients who are treated according to fixed administration schemes show a strong inter individual variation in serum ASNase levels. While underexposure may compromise therapeutic benefits, strongly elevated serum levels may lead to serious adverse effects including encephalopathy as a result of increased ammonia levels. Although ASNase levels and activity can be accurately monitored, in at least 10% of the patients the desired ASNase levels are either not reached or exceeded. **Aims**. Our understanding of ASNase dynamics *in vivo* needs to be improved. Here we report a link between a mutation in the gene encoding Cathepsin B and diminished ASNase clearance in a pediatric patient with ALL, suggesting a role for this protease in ASNase turnover. **Methods**. We have used cell biological and biochemical experiments to study the effect of a mutation in Cathepsin B that was found in a pediatric patient who experienced serious toxicity in reaction to ASNase treatment. **Results.** A 3-year-old girl diagnosed with ALL experienced serious toxicity in the form of hyperammonemic encephalopathy during treatment with ASNase derived from *Erwinia chrysantemi*. Pharmacokinetic data showed a strongly delayed clearance of the ASNase, which with the standard treatment protocol resulted in extremely high serum ASNase levels. Plasmapheresis was performed and administration frequencies were adjusted to improve the clinical status of the patient. No underlying metabolic disorder could be diagnosed.Based on a previous report describing the role of proteases in degradation of ASNase *in vitro*, we hypothesized that the cysteine protease Cathepsin B might be implicated in the strongly reduced clearance of ASNase in this patient. Sequencing of the open reading frame of the Cathepsin B gene (*CTSB*) revealed a single codon deletion in the germline of the patient, affecting a lysine residue in the carboxy terminus of the protein. Immunofluorescence and biochemical experiments show that this single amino acid deletion leads to a protein product that is retained in the endoplasmic reticulum and is inefficiently processed. ASNase degradation assays show that the mutant Cathepsin B has lost its ability to efficiently degrade ASNase which can explain the high levels of ASNase as observed in our patient. **Conclusions**. Together, these findings suggest that variation in Cathepsin B expression or activity may contribute to the large variation in serum ASNase levels in patients. Monitoring or therapeutically altering Cathepsin B activity could therefore improve ASNase administration regimens and therapy responses.

0525

TARGETING THE RAS/MAPK AND PI3K/MTOR SIGNALING PATHWAYS: A NEW APPROACH IN SENSITIVE AND RESISTANT CML CELL LINES?

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of the *BCR-ABL* fusion gene that encodes an oncoprotein, with a deregulated tyrosine kinase activity. The first-line treatment is Imatinib, a tyrosine kinase inhibitor that blocks BCR-ABL activity. This oncoprotein activates multiples signaling pathways responsible for tumor cells characteristic, namely the high cellular proliferation and resistance to apoptosis. One of these pathways is the RAS/MAPK that controls the cell proliferation and survival. The activation of RAS proteins requires the attachment of a farnesyl group, mediated by the farnesyltranferase. Other pathways include de activation of the PI3K/AKT/mTOR pathway. So, the knowledge of cell signaling pathways involved in CML may contribute to the development of new therapeutic approaches and also became an alternative in cases of resistance to standard therapies.The aim of this study was to evaluate the therapeutic potential of the farnesiltransferase and mTOR inhibitors (respectively, L-744832 and Everolimus) alone and in combination with Imatinib in sensitive and resistant CML cell lines.For this purpose, we used a CML cell line sensitive to Imatinib, the K562 cells, and established two sub-cell lines resistant to Imatinib, the K562-RC and K562-RD cells. To obtain these resistant cells, K562 cells were exposed to Imatinib following two strategies: continuous exposure of increasing concentrations of Imatinib (K562-RC cells) and discontinuous exposure, with interchanged mediums with and without Imatinib (K562-RD cells). To evaluate the effect of Imatinib, L-744832 and Everolimus in cell viability, all cell lines were treated in the absence and presence of different drug's concentrations and analyzed by the resazurin assay. Cell death was determined by optical microscopy (May-Grünwald staining) and by flow cytometry (FC) using the Annexin V/Propidium Iodide staining and by caspases expression levels. The effect of the drugs in cell cycle was accessed using Propidium Iodide incorporation by FC.Our results show that the half maximal inhibitory concentration (IC_{50}) of Imatinib in K562 sensitive cells was 75nM, whereas in K562-RC cells this value is increased eight times (605nM) and in K562-RD cells is around eighteen times higher (1389nM). The L-744832 shows a cytotoxic effect in sensitive and resistant cell lines, but the last one requires a higher dose (35mM vs 50mM, respectively). The same pattern was observed in presence of Everolimus, but this drug has also the ability of interfering with the cell cycle. The both compounds induced cell dead by apoptosis in a time- and dose-dependent manner. The association of lower doses of Imatinib (10nM) with 5uM of L-744832 or/and Everolimus shows a synergist cytotoxic effect in sensitive and in resistant cells. Combined therapy reveals to be a more effective strategy since the effects are greater with the use of smaller doses, even in cases of resistance.In conclusion, our results suggest that discontinuous drug exposure induces higher resistance levels. The inhibition of others pathways besides BCR-ABL oncoprotein could be used as a new potential approach in the treatment of CML and in overcoming resistance to standard therapies.Frist and second authors contribute equally to the study.

0526

EFFECTS OF AUTOPHAGY ON CYTOSINE ARABINOSIDE-HL60 INDUCED CELL DEATH

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Macroautophagy (autophagy) is a cellular process for degradation of proteins and defective organelles in eukaryotic cells, by their sequestration by double-layered membranes which form autophagosomes, which fuses with lysosomes where the sequestered contents are degraded. Studies have demonstrated that autophagy inhibition accelerates the demise of tumor cells that are subjected to chemo or radiotherapy, constituting an important target for the anticancer new strategies development. Cytosine arabinoside (AraC) is an important drug that has been used in the treatment of acute myeloid leukemia (AML) for more than three decades. However, only a few studies have demonstrated the signaling pathways involved in AraC-induced leukemia cell death and there are no reports involving the role of autophagy in the response of leukemia cells to AraC. In this work, we have evaluated the modalities of cell death induced by this drug alone and in association to the autophagy inhibitors 3-Methyladenine (3-MA) and Chloroquine (CQ) on human myeloid leukemia cell line (HL60). Cytotoxicity of AraC in association to 3-MA (10 mM) or CQ (25 μM) in HL60, assessed by trypan blue exclusion assay, demonstrated that 3-MA increased significantly the AraC effects to HL60 cells. AraC HL60-induced cell death, analyzed by flow cytometer in cells labeled with FITC Annexin V and PI, demonstrated an increase in the percentage of apoptosis and secondary necrosis in AraC/3-MA treated HL60 cells in relation to AraC alone. Interestingly, CQ, another autophagy inhibitor, when used in association to AraC, although it has also decreased the viability of HL60 cells and increased the percentage of cell death in relation to AraC alone, these data were not statistical different. The involvement of autophagy process in our results was evaluated by Western blot, which demonstrated an increase of p62 and decreased of LC3II/LC3I ratio in HL60 AraC treated cells in relation to control non-treated HL60 cells. No additional increase and decrease in these parameters (p62 and ratio LC3II/LC3I), respectively was observed in our results when HL60 cells were treated with 3-MA and CQ in association to AraC. Beclin-1 expression in HL60 cells was not modified by AraC alone or in association to autophagy inhibitors for 24 hours. Our results suggest that autophagy plays a complex role in the AraC-induced death of HL60 cells since it amplifies the AraC-mediated cell death programs. Thus, autophagy might be a mechanism of AraC resistance, since the pharmacological inhibition of this process sensitized HL60 cells to AraC death stimulus.**Financial Support:** FAPESP and CNPq.

SIMULTANEOUS SESSION I

Acute lymphoblastic leukemia - Biology

0527

ILLEGITIMATE V(D)J - MEDIATED RECOMBINATION IS LINKED TO GENDER BIAS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The incidence of childhood acute lymphoblastic leukemia (ALL) is higher by 20% in males relative to females. Male sex is also associated with a worse response to treatment and an inferior prognosis. The mechanism behind the phenomenon of gender-specific differences is presently not understood. **Patients, Methods and Results.** We have initially identified a previously described deletion of the 5' region of *C20orf94* - a gene coding for an uncharacterized DNA repair-associated protein - by SNP array and high-resolution CGH array in 5 of 20 ALL samples. As the breakpoints within *C20orf94* were defined to recurrent positions, we performed sequence analysis and revealed specific breakpoint junctions with typical characteristics of illegitimate V(D)J recombination. We next screened diagnostic leukemic specimens of a representative cohort of 512 patients enrolled into trial ALL-BFM 2000 by PCR. Here, *C20orf94* deletions were detected in 32.0%. The deletions were significantly associated with male gender ($P < 0.001$) and *ETV6/RUNX1*-rearranged ALL ($P < 0.001$). Additional screening of an independent cohort of 232 *ETV6/RUNX1*-rearranged ALLs identified 145 positive samples and not only confirmed the high incidence of *C20orf94* deletion in this subgroup, but also allowed validation of its specific association with male gender ($P = 0.049$). By analyzing additional 249 *ETV6/RUNX1* negative patients diagnosed in 2001 and 2002 the association with male gender was also confirmed in this subgroup ($P=0.029$). Breakpoint sequencing analysis in additional 38 patients confirmed illegitimate V(D)J recombination in all samples. In 21 of 22 paired initial and relapse leukemic samples, *C20orf94* deletions were maintained at relapse. Breakpoint analysis showed the same monoclonal sequences in nine patients indicating stability of *C20orf94* deletions during disease progression. The remaining samples either developed heterogeneity of *C20orf94* breakpoints at relapse subsequent to monoclonal sequences or already showed an oligoclonal pattern at initial diagnosis. We next collected information on *TAL1* deletion status - a second marker associated with illegitimate V(D)J-mediated recombination in childhood ALL. It occurs in approximately 5 to 15% of T-cell ALL. Employing the ALL-BFM 2000 trial and additional national trials conducted in Austria, Czech Republic, Israel and Italy, 128 (11.1%) *TAL1*-deleted patients were identified from an entire *TAL1* deletion-screened T-ALL population of 1149 patients. *TAL1* deletions were significantly more frequent in male patients ($P = 0.002$). **Conclusions.** As we found a clear association of *C20orf94* deletion with *ETV6/RUNX1*-rearranged ALL and male sex, our results not only suggest a potential role for *C20orf94* deletion and illegitimate V(D)J recombination in the pathogenesis of childhood ALL, but point towards gender-specific differences in illegitimate V(D)J-mediated recombination as one potential explanation for the observed and currently only poorly understood gender bias in childhood ALL.

0528

THE ORIGIN AND NATURE OF TIGHTLY CLUSTERED BTG1 DELETIONS IN PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA SUPPORT A MODEL OF MULTICLONAL EVOLUTION

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Background. B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common form of cancer in children and comprises genetically distinct subtypes. These ALL subtypes are characterized by specific genetic abnormalities, including aneuploidies (hyperdiploidy and hypodiploidy) and chromosomal translocations leading to *ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL1*, and *MLL* gene fusions. An additional layer of complexity was recently revealed by the identification of recurrent copy number alterations, including single gene deletions, which may act as 'drivers' in the development of BCP-ALL. One of the newly identified players in BCP-ALL is the *B cell translocation gene-1* (*BTG1*), which belongs to the *BTG/TOB* family of anti-proliferative genes and expression of their gene products is altered in a variety of different cancers. **Aims.** Recurrent deletions in genes affecting key cellular pathways are a hallmark of pediatric BCP-ALL. To gain more insight into the mechanism underlying these deletions, we have studied the occurrence and nature of abnormalities in *BTG1* in a large cohort of pediatric BCP- and T-lineage ALL cases. **Methods.** By performing SNP-based CGH analyses (n=24) and MLPA (n=831) clonal *BTG1* deletion breakpoint clusters were identified. Using a sensitive deletion-spanning PCR the deletion breakpoints were subcloned and further examined in detail for RAG recombination signal sequences and the presence of multiple subclonal *BTG1* deletions. Genomic sequencing of the open reading frame (n=293) and bisulphite sequencing of the *BTG1* promoter region (n=25) provided information about the presence of *BTG1* point mutations and promoter methylation, respectively. Finally, by chromatin immunoprecipitation (ChIP) the level of H3K4me3 and H3K9/14Ac within the *BTG1* gene locus was assessed to determine their role in the *BTG1* deletion mechanism. **Results.** *BTG1* was found to be exclusively affected by genomic deletions, which were detected in 65 out of 722 BCP-ALL patient samples (9%), but not in 109 T-ALL cases. There was no evidence for the presence of *BTG1* point mutations or promoter methylation. Eight different deletion sizes were identified, which all clustered at the telomeric site in a hotspot region within the second (and last) exon of the *BTG1* gene, resulting in the expression of truncated *BTG1* read-through transcripts. The presence of V(D)J recombination signal sequences at both sites of virtually all deletions strongly suggests illegitimate RAG1/RAG2-mediated recombination as the responsible mechanism. Moreover, high levels of H3K4me3, which is known to tether the RAG enzyme complex to DNA, were found within the *BTG1* gene body in BCP-ALL cells, but not T-ALL cells. *BTG1* deletions were rarely found in hyperdiploid BCP-ALLs, but were predominant in other cytogenetic subgroups, including the *ETV6-RUNX1* (19%) and *BCR-ABL1* (26%) positive BCP-ALL subgroups. Through sensitive PCR-based screening, we identified multiple additional *BTG1* deletions at the subclonal level in BCP-ALL, with equal cytogenetic distribution and which, in several cases, grew out into the major clone at relapse. **Conclusions.** Taken together, our results indicate that *BTG1* deletions may act as 'drivers' of leukemogenesis in specific BCP-ALL subgroups, in which they can arise independently in multiple subclones at sites that are prone to aberrant RAG1/RAG2-mediated recombination events.

0529

TLX HOMEODOMAIN ONCOGENES MEDIATE MATURATION ARREST IN T-ALL VIA INTERACTION WITH ETS1 AND SUPPRESSION OF TCRA GENE EXPRESSION

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Background. Acute leukemias are characterized by a multi-step oncogenic process leading to maturation arrest and malignant transformation of a hematopoietic precursor. TCR chromosomal translocations represent a recurrent oncogenic hallmark of T-ALL, amongst which over-expression of the homeobox proteins TLX1 and TLX3 are the most frequent. This oncogene deregulation is classically considered to be driven by the TCR enhancer. Almost all TLX1/3 T-ALLs are arrested at the cortical thymic stage, with expression of cytoplasmic TCRb but no surface TCRab. **Aims.** To determine whether TLX oncoproteins are linked to the cortical thymic maturation arrest and whether they impact on initiation, development and maintenance of TLX+ T-ALLs. **Methods.** An Ea-CAT gene reporter assay was used to analyse the role of TLX protein on Ea, including protein/protein interaction by CoIP and GST pull down followed by protein/DNA interaction by Chip and EMSA. The effect of TLX down-modulation was evaluated using ShRNA TLX1/3 in TLX+ cell lines. The effect of TCRab expression on differentiation and survival was also assessed. TCR-TLX1 translocations were analysed by FISH and LM-PCR. **Results.** The Ea-dependent reporter assay showed repression of Ea transcriptional activity by TLX1/3 proteins via ETS1. TLX1/3 and ETS1 proteins interact in the TLX1$^+$ ALL-SIL and TLX3$^+$ DND41 cell lines. The ETS1 protein led to a shift of the labelled Ea probe by EMSA, and addition of TLX1/3 produced a super-shifted complex. ChIP analysis demonstrated that knockdown of ETS1 reduced Ea-binding of both ETS1 and TLX1/3 in TLX$^+$ cell lines, in keeping with recruitment of TLX1/3 to Ea through ETS1 in TLX$^+$ T-ALL. These results suggested that inhibition of Ea activity by TLX1/3 in T-ALL blocks ab rearrangement and, consequently, T-cell maturation. Knock-down of TLX1/3 in TLX$^+$ cell lines induced sTCRab expression and massive apoptosis. ALL-SIL cells were transduced using lentiviral vectors enabling expression of TCRb and TCRa. sTCRab$^+$ ALL-SIL cells exhibited reduced viability and cell growth and increased apoptosis, when grown on OP9-DL1. These data demonstrate the key role of TCR expression and signalling in mediating cell death in TLX$^+$ T-ALLs, and strongly suggest that the apoptosis observed upon TLX inhibition is a consequence of re-differentiation.Mapping of the translocations breakpoints in *TCRb-TLX1* and *TCRd-TLX1* T-ALLs showed that whereas *TLX1* activation in *TCRb-TLX1* was consistent with classical TCR enhancer-mediated activation, the *TCRd-TLX1* translocations separated the *TLX1* gene and Ea on the two derivative chromosomes, indicating Ea-independent activation. TCRd-TLX1 translocations with breakpoints downstream to *TLX1* could however be found by PCR in normal thymus. This suggest that deregulation of *TLX1*, when driven in-*cis* by Ea, will not lead to oncogenic selection but rather to autonomous counter-selection of the chromosomal translocation due to feed-forward repression **Summary and Conclusions.** TLX1 expression in T-ALLs prevents TCRa rearrangement by ETS1 mediated inhibition of the TCR Ea, with consequent maturation arrest. Abrogation of this maturation arrest by loss of TLX expression or, more surprisingly, TCRab expression in an appropriate cellular context leads to cell death, providing evidence in favor of an oncogenic role for the failure to express a TCRab in TLX1 T-ALLs.

0530

LEUKEMIA INITIATING CELL: A NOVEL PROGNOSTIC MARKER IN B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Minimal residual disease (MRD) is currently the most powerful prognostic indicator in ALL. Using neonatal NOD/SCID/IL2rγ^{null}(NOG) mouse model, we previously reported that CD34$^+$CD38$^+$CD19$^+$ cells as well as CD34$^+$CD38$^-$CD19$^+$ cells have the capacities to initiate B-ALL *in vivo* and to self-renew, that is, CD34$^+$CD19$^+$ cells are candidate leukemia initiating cells(LICs) in human B-precursor ALL (B-ALL)(Kong Y et al. Leukemia 2008;22:1207-1213). Preliminary study demonstrated that mean fluorescence intensity of CD58 and CD123 were significantly higher on LICs from newly diagnosed B-ALL and relapsed B-ALL than that of normal B-cell progenitors (Kong Y et al. Blood 2011;118(21):1084a). Nevertheless, the delivery of the LICs identified in xenotransplantation assay to the clinics still remains controversial. **Aims.** To evaluate prognostic significance of the candidate LICs as a novel MRD biomarker in B-ALL patients. **Methods.** Using a cohort of 140 patients (including pediatric and adult patients) with CD34$^+$ B-ALL, candidate LICs were examined by seven-color flow cytometry (FCM) at different time points during treatment. A total of 1,050,000 events were routinely collected. More than 0.001% of LICs with aberrant overexpression of CD123 and/or CD58 in bone marrow samples after complete remission(CR) were defined as LIC positive (LIC+), all other cases were defined as LIC negative (LIC+). Real-time quantitative polymerase chain reaction (RQ-PCR) was applied concurrently to evaluate MRD in Ph$^+$ ALL, MLL$^+$ and TEL-AML1$^+$ patients. The value of the above specific genes equal to 0 was defined as RQ-PCR negative (RQ-PCR). The study was approved by the Ethics Committee of Peking University People's Hospital and written informed consent was obtained from all subjects. **Results.** The candidate LICs population with aberrant overexpression of CD123 and/or CD58 was identified in 100% of CD34$^+$ B-ALL patients at diagnosis. In the sequential MRD monitoring, sixty-four patients were detected as LIC+. Among them, LICs from 75% cases with CD58 overexpression, 97% with CD123 overexpression, and 100% with CD123 or CD58 overexpression. Thirty cases relapsed during follow up, of them 25 cases were detected as LIC+ at a median time of 57 days (13-90days) before the recurrence. In addition, a good correlation was found between the MRD results of LICs detected by seven-color FCM and RQ-PCR (n=313 pairs, Spearman r=0.889, p<0.001). Importantly, patients who were LIC+ after CR had a lower event-free survival (EFS) of 0.41 and a higher cumulative incidence of relapse (CIR) of 0.51 compared to an EFS of 0.90 and a CIR of 0.13 in LIC$^-$ patients (EFS, p<0.001; CIR, p<0.001). Similar results were obtained in high-risk patients and Ph$^+$ ALL patients. Multivariate analysis for EFS, overall survival (OS) and CIR showed that LIC+ after CR was an independent prognostic factor (p=0.000, p=0.000, and p=0.000, respectively). **Summary and Conclusions.** Detection of the B-ALL initiating cells with aberrant overexpression of CD123 and/or CD58 by seven-color FCM promises to be an efficient tool for MRD assessment and risk stratification in human B-ALL. **Acknowledgments.** This work was supported by grants from National Natural Science Foundation of China (30800483) and Beijing Municipal Science and Technology Program (Z111107067311070).

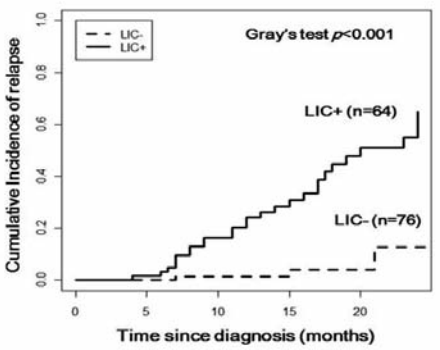

Figure 1

0531

AN AUTOMATED MICROSCOPY-BASED PLATFORM FOR FUNCTIONAL INVESTIGATION OF PROTECTIVE INTERACTIONS BETWEEN BONE MARROW STROMAL AND PRIMARY ALL CELLS

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Background. Leukemia cells are critically dependent upon interactions with the microenvironment in the bone marrow and extramedullary sanctuary sites, which is likely to provide a protective mechanism to escape the effect of chemotherapy. *In vitro*, co-culture of primary ALL cells on human mesenchymal bone marrow derived stromal cells (MSC) provides survival cues enabling long-term cultures. In contrast, primary ALL cells rapidly undergo cell death when cultured without stromal support. **Aim.** We developed an automated microscopy-based platform to identify pro-survival signals by RNA interference of candidate genes in MSC cells and subsequent evaluation on leukemia cell survival, enabling us to functionally profile primary leukemia cells. **Methods.** We took advantage of our leukemia xenograft system as a renewable source of well characterized samples derived from cases with very high risk ALL. Based on gene expression data and on cell surface proteomic data obtained from both cellular compartments, we generated a customized siRNA library for 110 candidate genes with a potential function in stromal support. Primary ALL cells were seeded on reversely transfected MSC cells in a 384 well format, and ALL cell viability was assessed with a fluorescent vital dye after 6 days. A machine learning algorithm was developed for quantification of surviving ALL cells on top of MSC. This readout correlated well with the standard method using 7-AAD by flow cytometry. **Results.** In this pilot study we evaluated three cases with VHR-ALL. We observed a consistent decrease of viability when interfering with the expression of 16 candidate genes. Interestingly the response patterns were different among the individual cases. As a proof of concept, we could show that down-regulation of VCAM1 and VEGFC had an effect on ALL survival as suggested by earlier studies. Down-regulation of VCAM-1 showed a decrease of ALL cell viability down to 58% compared to control on average for 3 different patients, whereas down-regulation of VEGFC decreased ALL cell viability down to 63%. One of the strongest effect on ALL survival by knockdown of a single gene was achieved for the membrane protein CD147 (Basigin). Down-regulation of CD147 decreased ALL cell viability in 12 of 17 patients tested. This protein exerts important chaperone function for monocarboxylate transporters, which have been shown to be important for tumor cell survival, and induces matrix metalloproteinases, which potentiate important microenvironmental signals. **Conclusions.** We have established a robust platform for systematic functional investigation of primary ALL cells in a 2-D model of the microenvironment. Critical interactions between ALL cells and bone marrow stromal cells can be identified with this approach, which can be extended for unbiased higher throughput screening and combinatorial testing. This platform will be of great interest for preclinical drug profiling on clinically relevant patients samples in the context of protective bone marrow signals. Finally, our data indicate that important differences will be found between leukemias derived from different patients, which underscores the need for a comprehensive study to better define functional subgroups and their potential clinical implications with respect to a more personalized approach to treatment.

Biological risk stratification in acute myeloid leukemia

0537

MINIMAL RESIDUAL DISEASE ASSESSED BY NPM1 MUTATION-SPECIFIC RQ-PCR ASSAYS HAS A PROGNOSTIC VALUE IN ACUTE MYELOID LEUKEMIA (AML) AND IS SIGNIFICANTLY DECREASED BY TREATMENT WITH GEMTUZUMAB OZOGAMICIN

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Background. *NPM1* gene mutations are frequently identified in AML. They have been reported as suitable molecular markers for minimal residual disease (MRD) monitoring. Recently, gemtuzumab ozogamicin (GO) has been shown to improve event-free and overall survival in patients with AML. **Aims.** To follow MRD in patients with *NPM1* mutated (*NPM1*mut) AML treated in the randomized ALFA-0701 GO trial conducted by the Acute Leukemia French Association (Castaigne et al, ASH meeting 2011), in order to evaluate its prognostic significance and the effect of GO on MRD level. **Methods.** The ALFA-0701 trial included 278 patients aged 50-70 years with newly-diagnosed *de novo* AML, randomized to receive or not five doses of 3 mg/m^2 GO, on day 1, 4 and 7 of standard induction chemotherapy and on day 1 of two consolidation chemotherapy courses, respectively. A total of 93 *NPM1*mut AML patients were studied (45 GO arm, 48 control arm). Peripheral blood and bone marrow samples were collected at diagnosis, after induction and after each consolidation course. MRD levels were assessed using cDNA-based real-time quantitative PCR (RQ-PCR) assays specific for the detection of the 3 most common types of *NPM1* mutations, A (n=72), B (n=10) and D (n=5) and reported as the ratio *NPM1*mut transcript/100 *ABL* transcript, with a threshold for MRD quantification at 0.1%. Occurrence of a MRD level below this threshold was defined here as a good MRD response. The prognostic value of MRD was assessed by comparing the outcome of good *versus* poor MRD responders after induction and after second consolidation, in terms of cumulative incidence of relapse (CIR). Effect of GO was then evaluated by comparing MRD levels in both treatment arms.

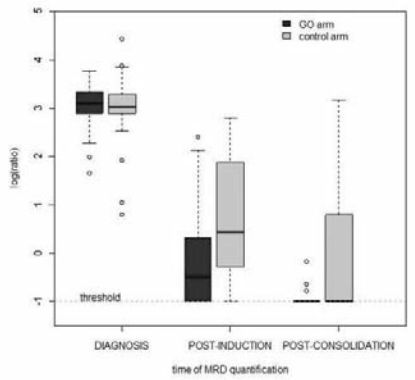

Figure 1. MRD levels according to treatment arm (GO or control).

Results. *NPM1*mut transcript levels at diagnosis were available for 77/93 patients (42 GO, 35 control) and had no prognostic impact on complete remission (CR) rate or CIR. In the 67 CR patients, post-induction MRD level was predictive of relapse: one-year CIR was 50% [95% CI: 36%-64%] in the 51 poor *versus* 27% [95% CI: 3%-52%] in the 16 good MRD responders (p=0.03). This prognostic impact on CIR was also observed for post-consolidation MRD level (SHR=0.3, 95%CI: 0.1 - 0.6; p= 0.002). Post-induction and post-consolida-

tion MRD levels were lower in the GO as compared to the control arm (Figure 1). The rate of good MRD response was 14/37 (38%) *versus* 2/30 (6%) after induction (p=0.004) and 24/27 (89%) *versus* 11/20 (55%) after consolidation (p=0.02), in GO and control arm patients respectively. Separate analysis in each treatment arm was limited by low patient numbers. **Conclusions.** Our data confirm the already reported predictive value of *NPM1* MRD and show that treatment with GO had a sustained effect on MRD reduction after induction and after consolidation.

0538

PROGNOSTIC IMPACT OF MINIMAL RESIDUAL DISEASE IN RUNX1-RUNX1T1 ACUTE MYELOID LEUKEMIA (AML): A STUDY OF THE GERMAN-AUSTRIAN- AML STUDY GROUP (AMLSG)

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Background. Acute myeloid leukemia (AML) with translocation t(8;21)(q22;q22) is a clinicopathologic genetic entity that is recognized in the WHO category of AML with recurrent genetic abnormalities. At the molecular level t(8;21) results in the *RUNX1-RUNX1T1* fusion gene which can be used as a molecular marker for monitoring minimal residual disease (MRD). In contrast to AML with *CBFB-MYH11*, *MLLT3-MLL* and *NPM1* mutation for which the prognostic impact of MRD monitoring could be demonstrated, the value of MRD in *RUNX1-RUNX1T1* positive AML is still undefined. **Aims.** To evaluate the prognostic impact of MRD in a large cohort of *RUNX1-RUNX1T1* positive AML patients by quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) during and after treatment. **Methods.** A total of 68 patients (age 18-60 years) were treated within one of the AMLSG treatment trials (AMLHD93 n=2, AMLHD98A n=12, AMLSG 07-04 n=54). Treatment consisted of double induction therapy with ICE (idarubicin, cytarabine, etoposide); postremission therapy comprised high-dose cytarabine-based regimens(n=59) as well as autologous (n=2) or allogeneic (n=4) stem cell transplantation (SCT) in first CR. Median follow up for survival was 43.5 months. Patient samples (bone marrow [BM] and/or peripheral blood [PB]) were collected at diagnosis (n=124), during treatment (n=354), and during follow up (n=305). RQ-PCR was performed using TaqMan technology. MRD levels were reported as the normalized values of *RUNX1-RUNX1T1* transcripts per 10^6 *beta-2-microglobulin* (*B2M*) transcripts (*RUNX1-RUNX1T1*/10^6 *B2M*), hereafter designated as *RUNX1-RUNX1T1* transcript levels. The maximum sensitivity of the assay was 10^{-6}. **Results.** At diagnosis transcript levels in BM samples ranged from $2{,}9x10^4$ to $14.4x10^6$ (median $3.0x10^5$) without correlation to presenting clinical features; in PB samples transcript levels ranged from $6.3x10^3$ to $10.3x10^6$ (median $2.3x10^5$) and were associated with white blood cell count (p=0.06) and peripheral blood blast percentage (p=0.02). There was no prognostic impact of pretreatment transcript levels in BM (HR, 1.07; p=0.86) and PB (HR, 1.02; p=0.96) on relapse free survival (RFS). After induction therapy the $\log_{10}$ reduction of transcript levels ranged from 1.4 to 6.6; three patients became MRD negative in BM and remained in sustained CR. During consolidation therapy 20 and 21 patients became at least at one time point MRD negative in BM or PB samples, respectively. This resulted in a significantly better RFS (p=0.02) of 85% (95%-CI, 70-100%), 65% (95%-CI, 47-90%) and 46% (95%-CI, 26-83%) in BM-negative, PB-negative and never-RQ-PCR negative (n=13) patients, respectively. The beneficial effect of RQ-PCR negativity translated into a better overall survival (p=0.008). After consolidation therapy, 21 of the 41 BM- and/or PB-negative patients became positive at least in one sample during follow up. Ten patients developed increasing transcript levels and all relapsed, whereas in the remaining 11 patients only single samples became positive with a maximum value of 881. **Conclusions.** In our study, achievement of RQ-PCR negativity after induction and consolidation therapy was significantly associated with favorable outcome in *RUNX1-RUNX1T1*-positive AML. MRD monitoring should be incorporated in future clinical trials to evaluate whether early intervention will improve outcome of the patients.

0539

GATA2 MUTATIONS ARE FREQUENT IN INTERMEDIATE RISK KARYOTYPE AML WITH BIALLELIC CEBPA MUTATIONS AND ARE ASSOCIATED WITH FAVORABLE PROGNOSIS

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Background. *GATA2* belongs to the *GATA* family of zinc finger transcription factors with importance in gene regulation in hematopoiesis. Recently, *GATA2* mutations have been described in families with predisposition to AML/MDS and in cytogenetically normal AML (CN-AML) with biallelic *CEBPA* mutations (*CEBPA*mut). **Aims.** The evaluation of *GATA2*mut in AML with intermediate-risk karyotype for frequency, association with other mutations and impact on outcome. **Methods.** We analyzed 212 cases with intermediate risk *de novo* AML for *GATA2*mut by direct Sanger Sequencing of exon 4 and 5, which contain the two highly conserved zinc finger domains of *GATA2.* Mutation status of other genes were available as follows: *NPM1*: n=212, *FLT3*-ITD: n=212, *CEBPA*: n=212, *MLL*-PTD: n=211, *RUNX1*: n=207, *ASXL1*: n=138, *FLT3*TKD: n=209, *WT1*: n=108. 156 cases (73.6%) were CN-AML and 56 (26.4%) had intermediate-risk aberrant cytogenetics according to MRC criteria. Female/male ratio was 107/105 and age ranged from 15.7-84.9 y (median: 59.7). **Results.** Overall, in 21/212 patients (9.9%) *GATA2*mut were detected. All mutations were point mutations. In detail, most mutations (n=14) were located in the first zinc finger domain (ZFD) (aa 294-344). Five patients harbored a mutation in the second ZFD (aa 349-398). One patient had two mutations in the first ZFD and one patient had each one mutation in each of the two ZFDs, respectively. In 10/21 *GATA2*mut cases remission samples were available and in all these cases the *GATA2*mut were not detectable in remission and thus were somatic and not germline. *GATA2*mut tended to be more frequent in females than in males (n=15/107, 14.0% vs 6/105, 5.7%, p=0.064). There was no association with age, leukocyte count, hemoglobin level, platelet count or cytogenetics. In 103 cases immunophenotyping data was available and cases with *GATA2*mut (n=10) had a higher expression of CD133 (52±29% vs 29±27%, p=0.015), CD34 (67±30% vs. 42±31%, p=0.018) and HLA-DR (59±28% vs. 38±24%, p=0.017) as well as lower expression of CD11b (19±14% vs 37±24%, p=0.003) and CD36 (10±5% vs. 23±15%, p<0.001) and thus had a more immature phenotype as compared to *GATA2*wt. With regard to cytomorphology we observed a preponderance of AML M1 (n=12/21) and AML M2 (n=8/21) subtypes in *GATA2*mut cases. In addition, *GAT*A2mut were strongly associated with *CEBPA* biallelic mutations (n=18/120, 15.0% vs 3/92, 3.3% in *CEBPA* wt, p=0.005), whereas *GATA2*mut were mutually exclusive of *CEBPA* monoallelic mutations (0/22) and of *FLT3*-ITD (0/45). Patients with *GATA2*mut had significantly better 2-year overall survival (2y-OS) (100% vs. 53.1%, p=0.001) and 2y event-free survival (63.8% vs. 35.7%, p=0.025) compared to *GATA2*wt cases. Because of the high coincidence of the two mutations the prognostic impact of *GATA2*mut in dependence of biallelic *CEBPA*mut was analyzed. Patients with biallelic *CEBPA*mut and additional *GATA2*mut (n=18) had a better 2ys-OS compared to patients with biallelic *CEBPA*mut and *GATA2*wt (n=80) (100% vs: 76.1% p=0.058). **Summary.** *GATA2* mutations are frequent in intermediate risk AML and are associated with female sex, biallelic *CEBPA*mut and favorable impact on survival. *GATA2* thus seems to be a promising new marker to identify patients with even more favorable prognosis in biallelic *CEBPA*mut AML.

0540

UNIQUE ASSOCIATION OF GATA2 ZINC FINGER 1 MUTATIONS WITH BIALLELIC CEBPA MUTATIONS IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA: FUNCTIONAL AND PROGNOSTIC RELEVANCE?

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Background. We and others have previously shown that cytogenetically normal acute myeloid leukemia (CN-AML) with biallelic *CEBPA* gene mutations (bi*CEPBA*) represents a distinct disease entity with a homogeneous gene expression profile and an independent favourable clinical outcome. Patients with bi*CEBPA* mutations are rarely associated with prognostic mutations commonly occurring in CN-AML, like mutated *NPM1*, *FLT3*-ITD, mutated *DNMT3A* or *IDH1/2*.So far, it is not known if other unknown genetic alterations cooperate with bi*CEBPA* mutations during leukemogenesis.**Aims**Therefore, we aimed to discover collaborating mutations in bi*CEBPA* mutant AML and to study their frequency as well as prognostic and functional relevance. **Methods.** We performed whole exome sequencing of five bi*CEBPA* patients at diagnosis and at remission. Leukemia-specific variants were verified by Sanger sequencing and their incidence evaluated in a larger cohort by scanning all coding exons using high resolution melting analysis. Mutation data were correlated to important clinical and molecular markers and to overall and event-free survival. The *in vitro* functional relevance of the most interesting mutations was tested using Dual Luciferase Reporter gene assays. **Results.** Somatic *GATA2* mutations were recurrently found in two out of 5 sequenced exomes. Further mutational screening uncovered *GATA2* mutations in 13 of 33 bi*CEBPA* positive CN-AML patients (39.4%). Interestingly, *FLT3*-ITD (6/33) was negatively associated with *GATA2* mutations in bi*CEBPA* mutant AML (p=0.027, Fisher's Exact Test).Strikingly, no *GATA2* mutations were found in 38 CN-AML patients with a monoallelic *CEBPA* mutation and in 89 CN-AML patients with wild-type *CEBPA* status. All *GATA2* mutations were exclusively located in the highly conserved N-terminal zinc finger domain (ZF1). The *GATA2* missense mutations A318T and G320D surrounding the C319 which coordinates the zinc atom were recurrently detected in 6 out of 13 bi*CEBPA* patients. *GATA2* mutations were lost during disease remission.Both GATA2 and CEBPA are transcription factors crucial for hematopoietic development. Inherited or acquired mutations in both genes have been associated with leukemogenesis. Interestingly, GATA2 is a direct protein interactor of CEBPA (Tong *et al*, MCB, 2005).In reporter gene assays using a CEBPA responsive promoter, all tested GATA2 ZF1 mutants showed reduced capacity to enhance CEBPA-mediated activation of transcription suggesting that the GATA2 ZF1 mutations may collaborate with biCEPBA mutations to deregulate target genes during malignant transformation. The presence of *GATA2* mutations did not negatively impact on the favourable overall and event-free survival of bi*CEBPA* patients. **Summary and Conclusions.** In summary, we describe for the first time the specific association of recurrent *GATA2* ZF1 mutations with bi*CEBPA* mutations. Our data suggest that GATA2 ZF1 and biCEBPA mutations functionally collaborate in interfering with the transcriptional activation of CEBPA.

0541

THE INCIDENCE AND PROGNOSTIC SIGNIFICANCE OF GATA2 MUTATIONS IN AML PATIENTS WITH CEBPA MUTATIONS

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GATA2 is a transcription factor that is important for megakaryocytic differentiation and the proliferation and maintenance of haematopoietic stem/progenitor cells. A number of recent studies have revealed germ-line mutations in the GATA2 gene in a range of phenotypically diverse disorders including familial MDS/AML (Hahn *et al*, Nat Genetics 43:1012, 2011), although studies of sporadic AML cases have reported either no acquired mutations (Zhang *et al*, PNAS 105:2076, 2008; Hahn *et al*, Nat Genetics 43:1012, 2011) or occurrence at low frequency (3.6%; Yan *et al*, Nat Genetics 43:309, 2011). However, a recent exome analysis of 5 AML cases with a normal karyotype (NK) and biallelic CEBPA mutations (CEBPA-double) detected recurrent acquired GATA2 mutations in these patients (Dufour *et al*, Blood 118:35, 2011). Strikingly, they found mutations in 41% of 32 CEBPA-double patients, all located in the N-terminal zinc finger domain of GATA2, but no mutations in 38 cases with one CEBPA mutation (CEBPA-single) or 90 NK patients with wild-type CEBPA (NK/CEBPA-WT). CEBPA-double patients have a favourable outcome compared to CEBPA-WT or CEBPA-single cases, although this benefit is lost in the presence of a FLT3/ITD (Green *et al*, JCO 28:2739, 2010). Given that the particular combination of mutations present in the same patient can influence outcome, we investigated the incidence of GATA2 mutations in CEBPA-mutated cases and analysed the impact on outcome in CEBPA-double patients. Exons 4, 5 and 6 of GATA2, encoding both zinc finger domains, were analysed by dHPLC using samples from 150 young adult AML patients treated on the UK MRC AML 10 or 12 trials. GATA2 mutations were detected in 14 of 53 (26%) CEBPA-double patients and also in 6 of 42 (14%) CEBPA-single cases, this difference not being significant (P = .15). No GATA2 mutations were found in 55 NK/CEBPA-WT patients. All mutations detected were located in one of the two zinc finger domains and they were predominantly missense mutations. The same mutations were detected in both CEBPA-single and CEBPA-double cases and recurrently affected A318 (n=6), L321 and R330 (both n=4). One CEBPA-double patient had two GATA2 mutations. There was a negative association between a GATA2 mutation and a FLT3/ITD in CEBPA-mutated cases, 0 of 20 (0%) GATA2-mutated cases were FLT3/ITD-positive vs. 16 of 75 (21%) GATA2-WT (P=.004), suggesting that these mutant genes have overlapping roles in the leukaemogenic process. Outcome analyses were performed for the CEBPA-double patients excluding the 6 FLT3/ITD-positive cases. GATA2-WT cases had a better remission rate than GATA2-mutant cases (97% vs. 79%, OR=10.1, 95% CI=1.10-92.7, P=.04). However, overall survival at 5 years was similar for the two groups (67% vs. 71% for GATA2-WT and GATA2-mutant, respectively; HR=0.71, CI=0.26-1.96, P =.5,) probably due to the lower, although not significantly different, relapse rate in the GATA2-mutant group (38% vs. 19%, HR=0.44, CI=0.15-1.30, P = .14). Therefore, although GATA2 and CEBPA mutations commonly occur together, there is no evidence that identification of the former provides additional prognostic information.

Chronic lymphocytic leukemia - Clinical

0542

THE BRUTON'S TYROSINE KINASE INHIBITOR IBRUTINIB IS HIGHLY ACTIVE AND TOLERABLE IN RELAPSED OR REFRACTORY (R/R) AND TREATMENT NAÏVE (TN) CLL PATIENTS, UPDATED RESULTS OF A PHASE IB/II STUDY

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Background. BTK is an essential mediator of B-cell receptor signaling and a critical kinase for lymphoma cell survival. Ibrutinib (PCI-32765), an oral, selective, irreversible inhibitor of BTK, inhibits proliferation, migration and adhesion in CLL cells. The combination of fludarabine, cyclophosphamide and rituximab have markedly improved outcomes of younger-fit patients in the first and second-line setting. However, effective salvage regimens for patients who develop treatment resistance are lacking. Fludarabine-based therapy, while effective is toxic and carries significant risk of morbidity and mortality in elderly pts. Patients who relapse following fludarabine-based regimens and older CLL patients represent a high priority for new therapeutic approaches. A multicohort Phase Ib/II trial evaluated 2 doses of single-agent ibrutinib in TN and R/R CLL/SLL patients**. Aims.** The primary objective was to determine the safety of 2 dose regimens. Secondary objectives were to assess the preliminary efficacy, PK, and long-term safety. **Methods.** Patients with R/R CLL who had failed at least 2 lines of therapy (including a purine analog) and TN CLL patients >65 years old were treated with oral ibrutinib at doses of 420mg or 840 mg administered daily for 28-day cycles until disease progression (PD).

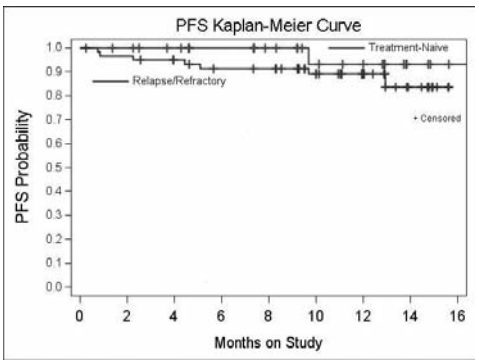

Figure 1.

Results. The data cut-off for R/R patients was 10/25/11 and 12/10/11 for TN patients. 61 patients were enrolled to the R/R cohorts- 27 patients (420mg) and 34 patients (840 mg). 31 patients were enrolled to the TN cohorts- 26 patients (420 mg) and 5 patients (840 mg). The 840mg TN cohort was terminated after comparable activity and safety between doses was shown in R/R patients. Median age was 64 and 71 years for R/R and TN patients respectively. 59% of R/R and 61% of TN patients had baseline cytopenias consistent with advanced stage disease. Unmutated IgVH was present in 79% of R/R and 43% of TN patients. Del17p was present 36% (R/R) and 6% (TN) of patients. The majority of AEs have been Gr<=2 in severity, most commonly diarrhea, nausea, and fatigue. Hematologic toxicity >= G3 was infrequent. Response (ORR; PR + CR) by IWCLL criteria in the 420 mg R/R cohort was 67% with 12.6 months median follow-up. In the 840 mg R/R cohort ORR is 68% at 9.3 months median follow-up. An additional 22%, and 24% of patients in these R/R cohorts, respectively, achieved a nodal response with residual lymphocytosis (NodR). With 10.7 months median follow-up the ORR in the 420mg TN cohort is 73% including 8% CRs with morphologically normal marrows. With only 4.6 mo median follow-up, ORR in the 840 mg TN cohort is 40%. An additional 12%, and 20% of patients in these TN cohorts, respectively, achieved a NodR. ORR was independent of high-risk factors. Estimated 12 month median PFS for those pts treated at 420 mg dose is 88% for R/R patients and 93.3% for TN patients. **Conclusions.** PCI-32765 is highly active and well tolerated in R/R and elderly TN CLL patients. No differences in activity or toxicity were noted between the two dose levels. The high ORR and very low PD rate indicate that ibrutinib warrants further study in CLL.

0543

THE BRUTON'S TYROSINE KINASE (BTK) INHIBITOR IBRUTINIB COMBINED WITH BENDAMUSTINE AND RITUXIMAB IS ACTIVE AND TOLERABLE IN PATIENTS WITH RELAPSED/REFRACTORY CLL, INTERIM RESULTS OF A PHASE IB/II STUDY

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Background. Bruton's Tyrosine Kinase (BTK) is an essential mediator of B cell receptor signaling and a critical kinase for lymphoma cell survival. Ibrutinib (PCI-32765), is an oral, selective, irreversible inhibitor of BTK, that inhibits proliferation, migration and adhesion in CLL cells. Single agent ibrutinib administered to relapsed/refractory CLL patients was highly active as evidenced by an IWCLL overall response rate (ORR) of 67% and estimated 12 month PFS of 86% (O'Brien ASH 2011). Bendamustine (B) and rituximab (R) (BR) is also active in relapsed/refractory CLL patients with an ORR of 59% and median PFS of 15 months (Fischer JCO 2011). We report interim data on the combination of ibrutinib with BR. **Aims.** The primary objective of the study is to evaluate the safety of ibrutinib in combination with BR administered to relapsed or refractory CLL patients. The secondary objective is to estimate the efficacy of the combination. **Methods.** Relapsed/refractory CLL patients received ibrutinib 420 mg orally daily for 28-day (D) cycles (C) until disease progression (PD). B was administered 70 mg/m^2 on D1 and D2 combined with R 375 mg/m^2 on D0 for C1 and 500 mg/m^2 on D1 for subsequent courses for a maximum of 6 cycles. Response was evaluated according to IWCLL criteria. **Results.** 30 patients were enrolled. The median age of patients was 62 yrs (range 41-82). 46% of patients were Rai stage III/IV and the median # of prior therapies was 2 (range 1-4). 37% and 13% were considered refractory (treatment free interval <12 mo) to a purine analog containing regimen or BR, respectively. Bulky disease (lymph nodes >= 5 cm) was present in 52% of patients. Adverse events (AE) have been generally consistent with that expected with BR. Gr 3/4 neutropenia and thrombocytopenia have been noted in 47% and 10% of patients, respectively. Grade >=3 non-hematologic AEs potentially related to ibrutinib included rash (3 pts) and fatigue and tumor lysis reported in 2 pts each. There were no Gr 3/4 infusion reactions. SAEs occurred in 10% of patients including one aforementioned case of TLS, one cellulitis, and one neutropenic fever. There have been no discontinuations due to AEs and no deaths on study. At a median follow-up of 4.9 mos (range 2.7-8.3 mo) 16 patients have completed BR (median 6 cycles, range 2-6) and 14 patients are still receiving BR. The ORR is 90% (27/30 patients) (CR 10%, PR 80%). 2 additional patients achieved a nodal response with residual lymphocytosis. Responses appear independent of high-risk features. 90% of patients remain on study; reasons for discontinuation include PD (n=2) and 1 pt pursuing SCT. **Conclusions.** Ibrutinib, administered in combination with BR, is safe and highly active. The high ORR, low rate of PD, and good tolerability compare very favorably with historical controls, warranting additional investigation of this combination.

0544

PHASE I STUDY OF THE CYCLIN-DEPENDENT KINASE (CDK) INHIBITOR DINACICLIB (SCH 727965) IN PATIENTS (PTS) WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. Dinaciclib (MK-7965/SCH 727965) is a novel, potent, small molecule inhibitor of CDKs 1, 2, 5, and 9 (IC_{50} values between 1-4 nM) with anti-leukemic activity against CLL. **Aims.** A Phase 1 trial was initiated to characterize the dose-limiting toxicity (DLT) and determine the recommended phase 2 dose (RPTD) of dinaciclib administered as a 2-hour IV infusion to patients with relapsed or refractory CLL. **Methods.** Patients received treatment on days 1, 8 and 15 of a 28-day cycle. Based on preclinical data, 5 mg/m^2 was selected as the initial starting dose. Once the RPTD was determined, additional patients were accrued utilizing an intrapatient dose escalation schedule to mitigate tumor lysis toxicity (7, 10 and 14 mg/m^2 on days 1, 8, and 15 during cycle 1 and 14 mg/m^2/dose for each cycle thereafter). Response was assessed according to 1996 NCI Working Group (NCI-WG) criteria for CLL. Patients were followed until disease progression, death, or dropout. **Results.** To date 39 pts have been treated in 5 dose-escalation cohorts (5, 7, 10, 14 and 17 mg/m^2). Median age is 62 (range 43-79) years, with 40% ≥65 years, 71% Rai stage 3 or 4, 63% with bulky disease, and 43% with del 17p. The median number of prior therapies is 3 (range 1-12) with 89% having received prior fludarabine. In the dose determination phase, the RPTD was established as 14 mg/m^2. Dose limiting toxicities (DLT) of bacterial pneumonia and tumor lysis syndrome were observed at the maximum administered dose of 17 mg/m^2. TLS was observed in 7 of the first 39 subjects with CLL, 2 of the 7 TLS events resulted in temporary hemodialysis. Both subjects recovered rapidly and continued dinaciclib after dose reduction. Other common treatment related adverse events included leukopenia, neutropenia, anemia, thrombocytopenia, hyperglycemia, and diarrhea. Clinical response as assessed by the 1996 NCI-WG CLL criteria indicated an overall partial response rate of 54%. Response rates in patients withdel 17p was 53% (9/17), and 58% (7/12) withdel 11. Overall clinical response rates on the RPTD were 69% (11/16), with 6 responders out of 8 patients with del 17. An additional 13 patients have been enrolled in the intrapatient dose escalation expansion cohort, of which 7 are currently evaluable for response. Five patients have achieved a PR, 1 stable disease and 1 with progressive disease. **Conclusions.** Dinaciclib demonstrates single-agent activity in heavily pre-treated CLL patients, including those with deletion 17p and other high-risk genomic features. Compared to first-generation CDK inhibitors, the drug demonstrates a broader therapeutic index, but investigation of alternative dosing schemes to further mitigate risk for tumor lysis are warranted and ongoing. This study supports further study of dinaciclib in CLL, both alone and in combination.

0545

NAVITOCLAX (ABT-263) PLUS FLUDARABINE/CYCLOPHOSPHAMIDE/RITUXIMAB (FCR) OR BENDAMUSTINE/RITUXIMAB (BR): A PHASE 1 STUDY IN PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. Orally bioavailable navitoclax binds with high affinity (Ki ≤1nM) to Bcl-2, Bcl-xL, and Bcl-w, promoting apoptosis. Preclinically, N enhances rituximab (R) efficacy in B-cell lymphoma, alone or combined with chemotherapy. Phase 1 data showed that N monotherapy was well-tolerated and had anti-tumor activity in CLL patients. **Methods.** We treated relapsed/refractory CLL patients in a phase 1 study to evaluate the safety and pharmacokinetics (PK) of escalating doses of navitoclax combined with standard-dose bendamustine (B)-rituximab (R) or a fixed dose of navitoclax (110 mg) with standard-dose fludarabine (F)-cyclophosphamide (C)-R. Secondary objectives were to assess efficacy endpoints (PFS, ORR, TTP, OS, duration of response). Patients required therapy as per iwCLL criteria and had ECOG ≤1. For patients treated with navitoclax-BR, navitoclax was administered once daily (starting dose, 110 mg) on D3-5 of C1 and D1-3 of subsequent cycles, with 6 patients per dose cohort. Dose escalations were via continuous reassessment to identify a dose combined with chemotherapy in which <33% of patients experienced dose-limiting toxicity (DLTs). We assessed tumor response (NCI-WG 1996 criteria; updated 2008) and adverse events (AE; NCI CTCAE V4). Patients received navitoclax for 1 year or until progressive disease (PD) or intolerable toxicity. **Results.** 26 patients (median age 58 yr [39-80]) enrolled in the BR navitoclax-dose escalation study and 5 patients in the fixed-dose navitoclax-FCR cohort. Median number of prior therapies was 2 (range 1-13). For patients treated with navitoclax-BR 5 had DLTs; 1 elevated liver enzymes (110 mg), 1 grade 4 febrile neutropenia (200 mg), and 3 grade 4 thrombocytopenia (250 mg). 1 patient had a DLT of febrile neutropenia in the navitoclax-FCR cohort. Frequent (>20%; any grade) AEs were nausea (77%), neutropenia (46%), fatigue (42%), vomiting (31%), pyrexia (31%), headache (31%) and diarrhea (27%). For patients treated with N-FCR, 2 had partial responses (PR) (including 1 with del[11q] CLL), 2 had stable disease (SD) and 1 had incomplete data. Among patients treated with navitoclax-BR, there were 6 complete responses (1 confirmed; 2 with del[17p] and del [11q] CLL), 7 PR (2 confirmed; 2 with del[17p] and del [11q] CLL and 1 with del[17p] CLL), 4 SD, 1 PD, and 8 with incomplete data.The overall response rate of patients treated with navitoclax-BR was 72% (13/18). Preliminary PK results suggest no apparent PK interaction between navitoclax and bendamustine. **Conclusions.** Navitoclax combined with BR appears well-tolerated and shows anti-tumor activity. The maximum tolerated dose (MTD) and recommended phase 2 dose of navitoclax is 250 mg. Navitoclax at 110 mg dose also appears well tolerated when combined with FCR. To date, unacceptable myelotoxicity has not been observed when navitoclax was combined with standard chemo-immunotherapy regimens for treatment of patients with CLL.

0546

SELECTIVE INHIBITION OF BCL-2 IS ACTIVE AGAINST CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): FIRST CLINICAL EXPERIENCE WITH THE BH3-MIMETIC ABT-199

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Background. New treatments are needed for patients with relapsed CLL, especially fludarabine-refractory disease. Targeted therapy with the BH3-mimetic navitoclax inhibits BCL-2, induces apoptosis and achieves partial remissions (PRs) in patients with CLL (Seymour EHA 2011). However, concomitant inhibition of BCL-x_L by navitoclax results in dose-limiting thrombocytopenia, highlighting the need for a more BCL-2-selective inhibitor. ABT-199 is a potent, orally bioavailable BCL-2 inhibitor (K_i<0.10 nM) with 500-fold less activity against BCL-x_L (K_i=48 nM). ABT-199 induces apoptosis of primary CLL cells *in vitro.* **Methods.** This first-in-human study of ABT-199 is a phase-I dose-escalation trial using a modified Fibonacci design in patients with relapsed/refractory CLL or non-Hodgkin lymphoma (NHL) with the objectives: evaluate safety, pharmacokinetics (PK), maximum tolerated dose, efficacy, and recommend a phase-2 dose. Patients with measurable disease, ECOG ≤1, and adequate marrow function were enrolled to Arm-A (CLL/SLL) or Arm-B (NHL). Data from Arm-A are reported. Patients received a single dose of ABT-199 on Week1 Day -7 (W1D-7), followed by continuous once-daily dosing from W1D1, until progressive disease (PD) or unacceptable toxicity. Evaluations included: adverse events (AE; NCI CTCAE V4), tumor response (NCI-WG 1996 criteria; updated 2008), and mechanism-of-action studies (Annexin-V and caspase-3 activation assays). **Results.** To date, 15 patients (median age 65 yr [36-84]) have enrolled. They had received a median of 3 (range 1-8) prior therapies; 8 had bulky adenopathy (≥5 cm), 8 were fludarabine-refractory, and 5 had del(17p) on FISH. The first three patients (cohort 1) experienced dose-limiting, clinically significant Gr3 tumour-lysis syndrome (TLS) following the W1D-7 single dose (200mg, 200

mg, 100 mg) of ABT-199, associated with a rapid reduction in palpable lymphadenopathy, and >95% reduction in lymphocytosis in the two subjects with pretreatment lymphocytosis. In the subsequent two cohorts, the W1D-7 dose was reduced to 50 mg, and the continuous dosing from W1D1 escalated weekly in step-wise fashion to the target cohort daily dose (150 mg [n=6], 200mg [n=6]). Gr3/4 ABT-199-related AEs observed include neutropenia (27%), TLS (27%) and febrile neutropenia (20%). Thrombocytopenia has not been dose-limiting. Preliminary PK was consistent with dose-proportional exposure. ABT-199 reached C_{max} approximately 6hrs after a low-fat meal, with a $t_{1/2}$ of ~21hrs. With a median follow-up of 4Mo, 11 patients are active; 4 have discontinued; 3 due to PD and 1 due to incidentally-detected adenocarcinoma. >50% reductions were observed in PB lymphocytes (11/11 patients with pretreatment lymphocytosis; median 86% [range 59-99%]), and lymphadenopathy (9/15 patients). In early formal response evaluations (W6 and W12), 8 (53%) patients had achieved PR (2 with marrow clearance); 4 confirmed, 4 awaiting re-assessment, including 4/8 F-refractory disease and 3/5 with del(17p). CLL cells collected 6-8hrs after initial dosing of ABT-199 show increased Annexin-V staining and caspase-3 activation. **Conclusions.** A single dose of ABT-199 can achieve a rapid reduction in CLL burden in patients with refractory disease. ABT-199 induces apoptosis of CLL cells *in vivo*, consistent with a mechanism-of-action based on BCL-2 inhibition. TLS can be mitigated with initial 50mg/day dosing and step-wise escalation. Patient accrual and identification of optimal dose and schedule for future studies continue.

Molecular advances in myelodysplastic syndromes

0547

MUTATIONS AFFECTING MRNA SPLICING DEFINE DISTINCT CLINICAL PHENOTYPES AND CORRELATE WITH PATIENT OUTCOME IN MYELODYSPLASTIC SYNDROMES

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Background. Mutations in genes encoding multiple components of the RNA splicing machinery in myeloid malignancies with myelodysplasia have been identified by next-generation sequencing approaches. The most frequently documented mutations affect splice genes *U2AF35*, Z*RSR2*, *SRSF2* and *SF3B1*. The high prevalence of these mutations suggests an important contribution of genetic alterations involving splice components to the pathophysiology of MDS. **Aims.** In the present study we investigated the clinical phenotype, the genetic characteristics and prognostic impact of *U2AF35*, Z*RSR2*, *SRSF2* and *SF3B1* mutations 221 MDS patients. **Methods.** DNA was extracted from diagnostic bone marrow cells. *SF3B1* (exons 12-16), *SRSF2* (exon2), *ZRSR2* (exons 1-11), and *U2AF35* (exons 2 and 6) were amplified using intron-flanking primers tagged with M13 universal primers at the 3' or 5' prime ends. PCR fragments were directly sequenced in both directions, and were analyzed using the Mutation surveyor version 3.97 software. Non-tumoral tissue was analyzed when available (DNA from buccal swab or CD3+ T-cells; n=20). In order to identify molecular interactions, the genomic regions that span exon 12 of *ASXL1,* exons 8 and 9 of *CBL*, exons 15-23 of *DNMT3A*, the entire coding regions of *ETV6* and *EZH2,* exons 4 of *IDH1* and *IDH2,* exon 14 of *JAK2*, exons 1 and 2 of *NRAS,* exons 3 to 8 of *RUNX1*, the entire coding region of *TET2,* and exons 5 to 8 of *TP53* were analyzed. Statistical analyses were performed using SPSS. **Results.** Among the 221 patients with MDS, 37 had *SF3B1* mutations (16.4%), 25 had *ZRSR2* mutations (11.1 %), 25 had *SRSF2* mutations (11.1%), and 12 patients harbored mutations affecting *U2AF35* (5.4%). In total, 99 mutations affecting one of the four genes were detected in 95 patients (42.2%). These mutations were mostly mutually exclusive and less likely to occur in patients with complex cytogenetics or *TP53* mutations. *SF3B1mut* patients presented with lower hemoglobin levels, increased WBC and platelet counts and were more likely to have *DNMT3A* mutations. *SRSF2*^{mut} patients clustered in RAEB-1 and RAEB-2 subtypes and exhibited pronounced thrombocytopenia. *ZRSR2*^{mut} patients clustered in IPSS int-1 and int-2 risk-groups, had higher percentages of bone marrow blasts and more often displayed isolated neutropenia. *SRSF2* and *ZRSR2* mutations were more common in *TET2*^{mut} patients. *U2AF35mut* patients had an increased prevalence of chromosome 20 deletions and *ASXL1* mutations. Multivariate analysis revealed an inferior overall-survival (OS) and a higher AML transformation rate for the genotype *ZRSR2*^{mut}/*TET2*^{wt} (OS: HR 3.3; 95%CI 1.4 - 7.7; *P*=.006; transformation: HR 3.6; 95%CI 2 - 4.2; *P*=.026). **Conclusions.** Our results demonstrate that splice gene mutations are amongst the most frequent molecular aberrations in MDS, they define distinct clinical phenotypes and show preferential associations with mutations targeting transcriptional regulation. Integration of molecular analysis of splice genes at diagnosis may improve the classification of MDS patients in the future.

0548

SPECTRUM OF GENE MUTATIONS AND THEIR INTRATUMORAL STRUCTURE IN MYELODYSPLASIA REVEALED BY HIGH-THROUGHPUT SEQUENCING

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Background. Myelodysplastic syndromes (MDS) are a group of myeloid neoplasms characterized by deregulated blood cell production and a high propensity to AML. To date, a number of gene mutations, which are largely common to many myeloid neoplasms, have been identified and implicated in the pathogenesis of MDS, including *RAS*, *TP53*, *RUNX1*, *c-CBL*, *TET2*, *ASXL1*, *EZH2*, *IDH1/2* and *DNMT3A*. Recently we reported comprehensive mutation analysis in 29 cases with myelodysplasia using whole-exome sequencing, which unmasked frequent mutations of the splicing machinery that is largely specific to MDS and other myeloid neoplasms with features of myelodysplasia. However, MDS are highly heterogeneous diseases and our knowledge about the spectrum of gene mutations with their intratumoral structure is still incomplete. **Aims.** The purpose of this study is to reveal the heterogeneity of MDS in terms of gene mutations and also characterize the intratumoral structure of those gene mutations in MDS and related myeloid neoplasms, using high-throughput deep sequencing in a larger set of MDS cases. **Methods.** To this aim, we performed whole-exome sequencing of additional 21 cases with MDS and related myeloid neoplasm and analyzed spectrum of gene mutations within a total of 50 myelodysplasia cases. We further analyzed a panel of 164 cases for recurrent mutations by target deep sequencing to accurately estimate frequency of each mutant allele within tumors. Whole genome sequencing was also performed in 5 cases to evaluate intratumoral structure of gene mutations. **Results.** Whole-exome analysis of 50 myelodysplasia cases disclosed a comprehensive registry of gene mutations in myelodysplasia, leading to the identification of a number of novel gene targets in myelodysplasia. Among these are *BCOR*, *STAG2* and other cohesine components. *BCOR* encodes an ubiquitously expressed nuclear protein which acts as a co-repressor of BCL6 and is recently reported to be mutated in acute myeloid leukemia. Cohesine is a multimeric protein complex, which regulates the separation of sister chromatids during cell division, post replicative DNA repair and regulation of gene expression. Subsequent mutation screening of these recurrently mutated genes for a panel of 164 MDS samples demonstrated these mutations were frequently mutated in MDS and other myeloid neoplasms. Mutation of cohesion complex occurred in an almost mutually exclusive manner among mutated cases. We also analyzed the allele frequencies of mutations in each tumor. Intratumoral heterogeneities of gene mutations seemed to exist in almost all cases, suggesting multiple clonal evolution events during the development of MDS.**Summary / Conclusions.** Whole exome analysis of 50 cases with MDS and related myeloid neoplasms revealed a full spectrum of common gene mutations in MDS including a number of previously unreported gene targets. MDS commonly shows intratumoral substructures, which may be implicated in the disease progression and evolutions.

0549

RECURRENT MUTATIONS OF MULTIPLE COMPONENTS OF COHESIN COMPLEX IN MYELOID NEOPLASMS

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BackgroundRecent genetic studies have revealed a number of novel gene mutations in myeloid malignancies. Many of these mutations, implicated in deregulated histone modification (*ASXL1* and *EZH2*) or DNA methylation (*TET2*, *IDH1/2* and *DNMT3A*), affected both acute and chronic myeloid neoplasms, indicating their general roles in myeloid leukemogenesis. However, our knowledge about the spectrum of gene mutations in myeloid neoplasms is likely to be still incomplete.**Aims**In the previous study, to obtain comprehensive complements of gene mutations in myelodysplasia, we analyzed 29 paired tumor-normal samples with chronic myeloid neoplasms with myelodysplastic features using whole exome sequencing. Although the major finding was the discovery of frequent spliceosome mutations uniquely associated with myelodysplasia phenotypes, a closer inspection of a revised list of mutations disclosed four candidate driver changes commonly affecting the cohesin complex, including *STAG2*, *STAG1*, and *PDS5B*. In this study, we aimed to confirm the finding from the discovery set and explore further the cohesin mutations in different myeloid malignancies.**Methods**We examined an additional 440 primary specimens of various myeloid neoplasms for gene mutations in major cohesin components, including *STAG1, STAG2, RAD21, SMC1A, SMC3* and *PDS5B*, using high throughput sequencing enhanced by DNA pooling. Copy number alterations in cohesin loci were also interrogated by SNP array karyotyping. **Results.** We identified a total of 38 Sanger-validated non-synonymous mutations and 16 deletions affecting 6 components of the cohesin complex in 52 out of the 469 primary cases with myeloid neoplasms (11%), including AML (17/115), CMML (13/84) and MDS (20/197), and also in 7 out of the 34 myeloid leukemia cell lines. 19 out of the 20 *STAG2*, 6 out of the 8 *RAD21* and one of the 5 *PDS5B* mutations were either nonsense, frameshift or splice site changes, which were predicted to cause a premature truncation of the protein. On the other hand, almost all the mutations found in *SMC1A, SMC*3 and *STAG1* were missense changes without showing apparent mutational hot spots. Most of the mutations and deletions were heterozygous and occurred in a mutually exclusive manner, suggesting that mutations in multiple cohesin components at a time may be catastrophic to cell viability.Unexpectedly, all 41 informative cases with cohesin mutations/deletions in our cohort showed diploid or near diploid karyotypes, of which 20 retained completely normal karyotypes, clearly arguing against the previous view that aneuploidy caused by these mutations would be responsible for their oncogenic action in glioblastoma and colorectal cancers. Alternatively, a growing body of evidence suggests that cohesin regulate gene expression, arguing for the possibility that cohesin mutations might participate in leukemogenesis through deregulated gene expression. Of additional note is that 6 cohesin-mutated cases accompanied significantly increased numbers of non-silent mutations in whole exome analysis, compared to non-mutated cases, raising the possibility that compromised cohesin function could induce DNA hypermutability associated with increased double strand break and contribute to leukemogenesis. **Summary and Conclusions.** In conclusion, massively paralleled sequencing of myeloid neoplasms revealed frequent mutations/deletions in cohesin complex. Our findings highlight a possible role of compromised cohesin functions in myeloid leukemogenesis.

0550

EFFECT OF SF3B1 MUTATION ON ERYTHROID MARROW ACTIVITY, TRANSFUSION IRON OVERLOAD, AND HEPCIDIN LEVELS IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background. The vast majority of patients with myelodysplastic syndrome (MDS) present with anemia, and most of them become transfusion dependent in the long term.The redistribution of transfusion iron from reticuloendothelial to parenchymal cells is modulated by hepcidin. In beta-thalassemia syndromes, ineffective erythropoiesis is associated with a downregulation of hepcidin, resulting in increased iron absorption and parenchymal iron loading.Different pathogenetic mechanisms are responsible for anemia in MDS. Recently, somatically acquired mutations in *SF3B1*, a gene encoding a core component of RNA splicing machinery, were identified in diverse WHO categories but predominantly in MDS patients with ring sideroblasts. The available evidence supports a causal relationship between *SF3B1* mutations and mitochondrial iron overload, and establish a link between these mutations and ineffective erythropoiesis. **Aims.** We studied *SF3B1* mutations, erythroid marrow activity, transfusion iron overload and hepcidin levels in patients with MDS in order to investigate the relationship between *SF3B1* mutation, ineffective erythropoiesis and parenchymal iron loading in these disorders. **Methods.** We evaluated 76 patients with MDS or myelodysplastic/myeloproliferative neoplasms (MDS/MPN) according to WHO criteria, followed at the Department of Hematology Oncology, University of Pavia & Fondazione IRCCS Policlinico San Matteo,Pavia,Italy.The coding exons of *SF3B1* were screened using massively parallel pyrosequencing. Erythroid activity was evaluated through soluble transferrin receptor (sTfR) and growth differentiation factor 15 (GDF15). Serum hepcidin-25 measurements were performed by combination of weak cation exchange chromatography and time-of-flight mass spectrometry.*Results*. Overall, 21 of 76 patients (28%) had a somatic mutation of *SF3B1,* with a median value for mutant allele burden of 44.7%. Significant associations were found between *SF3B1* mutation and proportion of bone marrow erythroblasts (*P*=.009), and sTfR levels (*P*=.039). Significant positive correlations were also found between these parameters and *SF3B1* mutant allele burden (*P*=.003, and *P*=.02, respectively). A significant effect of *SF3B1* mutation on GDF15 levels was noticed, mutant patients having higher values than wild-type ones (*P*=.023).Serum hepcidin ranged from 0.18 to 92.05 nM, and higher levels were found in transfusion-dependent versus transfusion-independent patients (*P*<.001). Multivariable analyses showed that hepcidin levels were independently determined by sTfR (the higher sTfR, the lower hepcidin; *P*=.015), serum ferritin (the higher serum ferritin, the higher hepcidin; *P*<.001), and *SF3B1* mutation (the higher allele burden, the lower hepcidin; *P*=.029). **Conclusions.** These findings provide evidence that *SF3B1* mutations are strongly connected with an expanded but ineffective erythropoiesis, resulting in inappropriately low hepcidin levels that may favor the internal redistribution of transfusion iron from macrophages to parenchymal cells.

0551

RAP-536 ATTENUATES INEFFECTIVE ERYTHROID DIFFERENTIATION AND REDUCES ANEMIA IN A MURINE MODEL OF MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes (MDS) are a complex group of hematopoietic stem cell disorders. Patients develop peripheral blood cytopenias such as anemia, neutropenia or thrombocytopenia and a significant proportion of patient progress to acute myeloid leukemia (AML). The erythroid hyperplasia, with increased rates of apoptosis and abortive maturation of erythroid precursors, leads to ineffective erythropoiesis and severe anemia. Despite high physiological levels of endogenous erythropoietin (EPO), patients with MDS are given recombinant erythropoietin therapy to stimulate erythropoiesis. Consequently, only 20%-25% of MDS patients respond to EPO therapy for their anemia. Therefore, alternative therapies to EPO that promote effective erythroid differentiation are necessary to treat anemia in MDS patients. Members of the TGFβ superfamily of signaling molecules have been implicated in erythropoiesis. ACE-536 is a soluble receptor fusion protein consisting of a modified activin receptor Type IIB extracellular domain linked to a human Fc domain. ACE-536 acts as a ligand trap to modulate the activity of TGFβ ligands and promote erythroid differentiation. While it is known that EPO treatment increases proliferation of erythroid progenitors, differentiation analysis of bone marrow and splenic erythroblasts after treatment with RAP-536 (murine version of ACE-536) demonstrated that ACE-536 promotes maturation of terminally differentiating erythroblasts.In this study we investigated the effect of ACE-536 on anemia during various stages of disease using a murine model of MDS. The NUP98-HOXD13 (NHD13) transgenic mouse carries a common translocation found in MDS patients. NHD13 mice develop anemia, neutropenia and lymphopenia starting at 4 months of age, with normal or hyper cellular bone marrow and majority (60%) of the mice progresses to AML over time. Over 90% of the mice die by 14 months due to severe pancytopenia or acute leukemia. In this study, mice were divided into three groups based on age. Early (~4 months old), mid (~8 months old) and late stage (~10 months) groups were randomized and dosed subcutaneously with either RAP-536 at 10 mg/kg or vehicle control twice per week for 8 weeks. Wild-type littermate controls were also dosed on the same schedule.All NHD13 mice in each group had severe anemia characterized by reduced RBC, Hemoglobin and HCT and compared to wild-type littermates at baseline prior to treatment. Treatment of RAP-536 for 8 weeks significantly increased RBC parameters and reversed anemia at all stages. Peripheral blood smear analysis revealed no indication of increased leukemic progression due to RAP-536 treatment. Cell differential and flow cytometric evaluation of erythroid precursors from bone marrow demonstrated decreased erythroid precursors and hyperplasia consistent with RAP-536 treatment compared to vehicle treated control.Our data demonstrate that RAP-536 can increase hematology parameters by enhancing maturation of terminally differentiated red blood cells. We have also shown RAP-536 corrects ineffective erythropoiesis, decreases erythroid hyperplasia and normalizes myeloid: erythroid ratios without enhanced progression to AML. Therefore ACE-536 may represent a novel treatment for anemia associated with MDS, particularly in patients that are refractory to EPO therapy. ACE-536 is currently being evaluated in Phase I clinical trials in healthy human volunteers.

Multiple myeloma - Biology

0552

A HIGH-RISK SURVIVAL SIGNATURE FOR MULTIPLE MYELOMA

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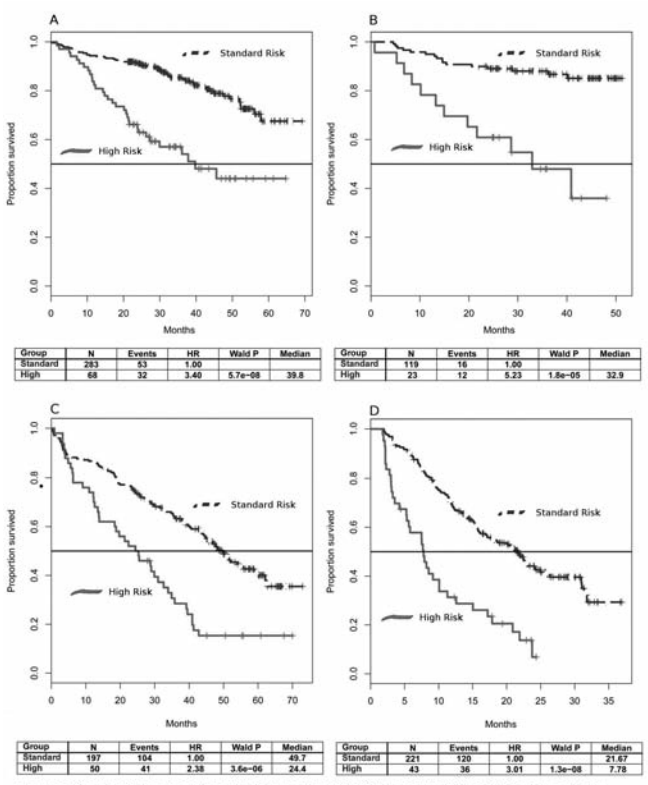
Background. Survival of patients with newly diagnosed multiple myeloma is highly variable and there is a strong need to improve current prognostic markers. Gene expression profiles reflect the biology of MM in individual patients and have potential to be of prognostic value.**Aims.**We aimed to establish and evaluate a prognostic signature based on gene expression profiling. **Methods.**The HOVON65/GMMG-HD4 trial compared the efficacy of bortezomib induction and maintenance treatment with standard induction and maintenance regimes.[1] For 290 patients, both gene expression and survival data were available. These were selected for training. Supervised principal components analysis was applied to generate a signature of 92 probe sets. High-risk disease within the training set was defined to be the proportion of patients with an overall survival of less than two years. Four independent datasets were available for validation, including newly diagnosed patients (TT2, n=351; TT3, n=142; MRC-IX, n=247) and relapsed patients (APEX, n=264).[1-5] **Results.** In all validation sets, patients defined as high-risk by the EMC-92-gene signature show a clearly reduced overall survival. A hazard-ratio (HR) of 3.4 (95%CI:2.19-5.29) was found for the TT2 study, HR:5.23 (2.46-11.13) for the TT3, HR:2.38 (1.65-3.43) for the MRC-IX and HR:3.01 (2.06-4.39) for the APEX (p<0.0001 in all studies; Figure 1).



Group	N	Events	HR	Wald P	Median
Standard	283	53	1.00		
High	68	32	3.40	5.7e−08	39.8

Group	N	Events	HR	Wald P	Median
Standard	119	16	1.00		
High	23	12	5.23	1.8e−05	32.9

Group	N	Events	HR	Wald P	Median
Standard	197	104	1.00		49.7
High	50	41	2.38	3.6e−06	24.4

Group	N	Events	HR	Wald P	Median
Standard	221	120	1.00		21.67
High	43	36	3.01	1.3e−08	7.78

Figure 1. Kaplan-Meier overall survival curves for EMC-92 signature defined high-risk patients versus standard-risk patients in four validation sets. (A) UAMS Total Therapy 2. (B) UAMS Total Therapy 3. (C) MRC-IX. (D) APEX. N, number of patients; Events, number of events; HR, hazard ratio; Wald P, p value for equality to standard-risk group; Median, median survival time.

Figure 1. Kaplan Meier curves in external data.

In multivariate analyses, the EMC-92-gene signature proved an independent and superior predictor compared with clinical variables such as the International Staging System and cytogenetic aberrations including del(17p). Furthermore, in standard-risk classified patients in the MRC-IX validation set, no survival difference was found between patients with or without at least one poor risk FISH marker, confirming the strength of the EMC-92 gene signature in relation to cytogenetics. In addition the performance of the EMC-92 is independent of existing signatures like the UAMS-17,70 and 80. Likelihood-ratio tests for pair-wise comparison showed that the increase in likelihood on addition of the EMC-92 was consistently higher than when another signature was added to EMC-92. For instance, the adjusted p-value for the addition of the EMC-92 to UAMS-80 and vice versa were $7.6x10^{-7}$and $2.2x10^{-3}$ respectively. The genes in the EMC-92 signature had little overlap to other signatures and was limited to genes such as *BIRC5* and *ITM2B*. Within the HOVON-65/GMMG-HD4 high-risk patients, bortezomib treated patients survived longer than patients treated with vincristine based chemotherapy (30 months compared to 19 months, respectively), albeit not significant (p=0.06). **Summary and Conclusions.** The EMC-92-gene signature has a strong and highly significant predicting ability in multiple myeloma patients irrespective of age and setting, newly diagnosed or relapse. Use of this signature will contribute to risk assessment in clinical trials and could ultimately provide a tool for treatment choices in high-risk multiple myeloma patients.This work was supported by the European Hematology Association, the Center for Translational Molecular Medicine, Skyline Diagnostics, Erasmus MC Translational Research Grant, Janssen and the FW6 program MSCNET.

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CIRCULATING MICRORNA IN MULTIPLE MYELOMA: DIFFERENCES WITH HEALTHY SUBJECTS AND CORRELATION WITH BIOLOGICAL PARAMETERS

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Background. microRNAs (miRNAs) are small non-coding RNAs able to regulate protein levels by binding mRNAs. In multiple myeloma (MM), miRNAs play a critical role in deregulating several pathways involved in MM pathogenesis. Since miRNAs are present in many body fluids with high stability, they hold great potential of being used as biomarkers. However, a comprehensive profile of circulating miRNA in MM patients and a comparison with healthy subjects is still lacking. **Aims.** To identify circulating miRNAs specific to MM patients and evaluate their association with biological characteristics. **Methods.** On 164 samples (104 from newly diagnosed MM patients and 60 from age-matched healthy subjects), a screening analysis of 654 miRNAs was performed to identify those consistently expressed using nCounter technology (nanoString, Seattle, USA). Expression of miRNAs present in at least 20% of samples has been confirmed by quantitative PCR (qPCR) performed on ABI7900HT with 2-ΔCt approach, using synthetic spiked-in miR-759 as endogenous control. miRNA marker levels were log2 transformed and linear regression model was used for comparison employing two-sample t-tests and Spearman rank correlation coefficients. **Results.** Median age of MM patients was 71 yrs (range 56-86) compared to 73 yrs (range 55-88) of healthy subjects. Within MM patients, beta2-microglobulin median value was 4,66 mg/l (range 1,2-12,96) and, according to ISS, 16 patients were in stage 1, 39 in stage 2 and 28 in stage 3. Median bone marrow infiltration by malignant plasma cells (%BMPC) was 60% (range 12-95). According to FISH status, 70 patients had standard and 29 high risk. Twenty-five miRNAs were expressed in at least 20% of the 164 analyzed samples. After qPCR analyses, 11 out of 25 miRNAs were expressed with a median Ct value lower than 35 cycles. A general reduction of these 11 circulat-

ing miRNA has been found in MM samples in comparison with healthy subjects: in particular six miRNAs (miR-92a, miR-451, miR-16, miR-19b, miR-25 and miR-30a) were statistically lower in MM as detailed in Table 1A. Focus on MM patients, univariate analysis showed correlation between specific miRNAs and predictors of poor prognosis. In particular, the expression of miR-92a, miR-19b, miR-21, miR-126 and miR-223 was inversely correlated with ISS stage, while miR-21 and miR-126 were correlated with high beta2-microglobulin values. An inverse trend was also observed between levels of some miRNAs and FISH risk, but without reaching statistical significance (model p-value in Table 1B). A clear correlation between low hemoglobin (Hb) levels and low expression of several miRNAs was also observed. Interestingly, no correlation was found between miRNA levels and %BMPC suggesting a correlation with organ damage but not with tumor load. **Conclusions.** This is the largest analysis of miRNA expression in serum of MM patients. A diffuse reduction in miRNAs levels was observed in MM patients compared with healthy subjects and a low expression of specific miRNAs correlates with adverse prognostic factors in MM patients. These observations strongly demonstrate a specific profile of circulating miRNAs in MM, opening the discussion on their role in the pathogenesis of the disease and their possible use as a biomarker.

Table 1.

A

miRNA	Healthy (n=60) median (range)	Myeloma (n=104) median (range)	P-value
miR-92a	0.05 (0.005 – 0.49)	0.02 (0.0005 – 0.28)	<0.00001
miR-451	0.045 (0.0015 – 0.8)	0.01 (0.0003 – 0.15)	<0.00001
miR-16	0.04 (0.00075 – 0.17)	0.02 (0.0003 – 0.22)	0.009
miR-19b	0.004 (0.0003 – 0.075)	0.002 (0.00004 – 0.09)	0.01
miR-25	0.0026 (0.00016 – 0.014)	0.0014 (0.00003 – 0.01)	0.004
miR-30a	0.0017 (0.0002 – 0.012)	0.0013 (0.00002 – 0.12)	0.0015

B

	miR-92a	miR-451	miR-16	miR-19b	miR-21	miR-25	miR-30a	miR-126	miR-223
ISS stage	**0.026**	0.087	0.13	**0.02**	**0.027**	0.058	0.058	**0.042**	**0.014**
% BMPC	0.59	0.28	0.89	0.5	0.91	0.73	0.94	0.19	0.083
b2-m	0.098	0.27	0.16	0.06	**0.034**	0.057	0.065	**0.033**	0.06
Hemoglobin	**0.003**	**0.0055**	0.068	**0.00038**	**0.0028**	**0.0033**	**0.005**	**0.023**	**0.029**
FISH risk	0.055	0.11	0.07	0.08	0.79	0.3	0.35	0.97	0.91

Fig 1: (A) comparison of miRNA expression in serum of healthy subjects and MM patients. P-value is based on univariate linear model with log transformed miR marker. **(B)** P-values from univariate models looking at factors and their correlation with log transformed miR markers

0554

STROMA-DERIVED EXOSOMES MEDIATE ONCOGENESIS IN MULTIPLE MYELOMA

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Background. Bone marrow (BM)-derived mesenchymal stromal cells (MSCs) support multiple myeloma (MM) cell growth, but little is know about the putative mechanisms that may regulate the interaction between clonal MM plasma cells and the surrounding BM milieu. We characterized the role BM-MSC-derived exosomes as key regulators of MM pathogenesis. **Aims.** 1) To determine the ability of BM-MSCs to release and transfer exosomes to MM cells. 2) To determine the role of BM-MSC-derived exosomes in inducing MM tumor growth and MM cell dissemination. **Methods.** MSCs were collected from BM of healthy subjects and MM patients, showing a multipotent MSC phenotype (CD34-/14-/45-/19-/138-;CD73+/90+/105+/106+). Exosomes were collected from conditioned-medium of normal-BM-MSCs and MM-BM-MSCs, or HS-5 cells; and studied using electron microscopy, immunogold-labeling, and western-blot for CD63 and CD81 detection. Transfer of PKH67-fluorescently-labeled-exosomes to MM cells was evaluated by time-lapse confocal microscopy. Transfer of murine-derived miRNA-containing exosomes into human MM cells was evaluated by qRT-PCR (exosomes were collected from BM-MSCs of C57BL/6 miRNA-15a/16-1-/- or C57BL/6 mice). miRNA expression profiling was obtained from normal (n=4) and MM (n=9) BM-MSCs-derived exosomes (TaqMan-human-miRNA-profiling). Normal and MM BM-MSCs-derived exosomes were loaded into tissue-engineered bones (TEB) with MM.1S-GFP+/Luc+cells: MM cell homing and MM tumor growth have been tested *in vivo* by using confocal-microscopy and bioluminescence-imaging (BLI), respectively. Loss- and gain-of-function studies were performed using normal-BM-MSCs, MM-BM-MSCs and HS-5 cells transfected with either pre- or anti-miRNA-15a. **Results.** Normal-BM-MSCs and MM-BM-MSCs released CD63+/CD81+ exosomes, as confirmed by electron microscopy, immunogold labeling, and western blot. BM-MSCs exosomes are transferred into MM cells, as shown by confocal microscopy; and further validated by qRT-PCR in human MM cell lines incubated with murine (C57BL/6 miRNA-15a/16-1-/- and wild-type) BM-MSCs-derived exosomes. The impact of normal-BM-MSC- and MM-BM-MSC-derived exosomes on MM cell behavior *in vivo* was next evaluated. MM cells co-cultured with MM BM-MSC-derived exosomes induced rapid tumor growth at the site of the TEB scaffold, as well as rapid dissemination to distant BM niches, as compared to MM cells co-cultured with exosomes derived from normal BM-MSCs. We next performed miRNA expression profiling on exosomes isolated from MSCs, and found increased expression of 24 miRNAs and reduced expression of 3 miRNAs in MM-BM-MSCs-derived exosomes versus normal (1.5 fold-change; P<0.05). Specifically, miRNA15a was significantly lower in MM-BM-MSC-derived exosomes, similarly to primary MM cells that present with reduced miRNA-15a expression. We therefore sought to examine whether lack of transfer of the tumor suppressor miRNA15a can lead to significant change in tumor growth and dissemination in MM, and found that by over-expressing miRNA-15a in normal BM-MSCs and HS-5 cells inhibited MM cell proliferation and adhesion to fibronectin. Next MM cells were cultured in presence of BM-MSCs isolated from either C57BL/6 mice or C57BL/6 miRNA15a/16-/: miRNA15a-deficient BM-MSCs significantly induced MM cell proliferation (P<0.05). Moreover, exosomes isolated from HS-5 pre-miRNA15a-transfected cells both inhibited MM cell proliferation and reduced their adhesion properties. **Conclusions.** These findings demonstrate the existence of exosome-driven interactions between the BM milieu and MM cells, suggesting that exosomes might constitute a novel mechanism for intercellular transfer of miRNAs to MM cells.

0555

IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS IN GENES INVOLVED IN MIRNA NETWORK ON SURVIVAL AND TOXICITIES IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA TREATED WITH BORTEZOMIB

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Background. A group of genetic normal variations in DNA, mainly single nucleotide polymorphisms (SNPs), have been described in association with prevalence, response to treatment, progression-free and overall survival (OS) in multiple myeloma (MM). A distinctive and relative new group of polymorphisms is constituted by SNPs in microRNAs (miRNA) processing machinery in miRNA precursor molecules and in miRNA binding sites, known as miRSNPs. The aim of this study was to ascertain the prognostic impact of 9 miRSNPs located either in MM related miRNAs target genes or in miRNA biogenesis pathway proteins, in order to correlate our findings with response, toxicities and OS to bortezomib in patients with relapsed myeloma. **Patients and Methods.** Seventy-five patients (37M/38F; median age 65 years, range 29 to 80) with relapsed MM were treated from December 2002 to March 2010 with bortezomib-based regimens. Median follow-up for patients alive was 31 months. Genomic DNA was isolated from bone marrow slides using a commercial kit (Qiagen). The genes and SNPs evaluated in genomic DNA by allelic discrimination (TaqMan assays) were *KRT81* (rs3660), *FAM179b* (rs1053667), *MIR146A* (rs2910164), *MIR196A2* (rs11614913), *MIR149* (rs2292832) and *MIR423* (rs6505162) for miRNA target genes, and *RAN* (rs14035), *TRBP* (rs784567) and XPO5 (rs11077) for miRNA biogenesis pathway. These genes were selected based on their potential impact on prognosis in solid tumors in previous reports.

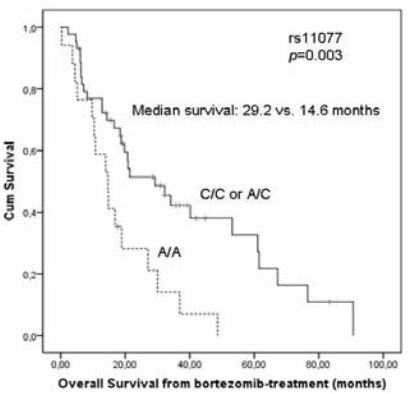

Figure 1. Overall survival according to the presence of polymorphism rs 11077 in XP05 (C/C=SNPs, A/C=herefozygous SNP, A/A=wild type).

Results. Overall response (OR) was achieved in 62% of the patients (complete remission 6.7%, partial response 44% and minor response 10.7%), while 9 (12%) and 20 (26.7%) showed no response (NR) or progressive disease (PD), respective-

ly. The median OS after bortezomib therapy was 19.6 months. OS was significantly longer in patients with SNP in *XPO5* (rs; *p*=0.003) (Figure 1) and *MIR146A* (0.037), with a trend for *TRBP* (0.086). *XPO5* rs11007 retained its prognostic impact on OS when a Cox multivariate regression analysis, including age (>65 years-old) and bone marrow plasma cell infiltration (≥50%), was performed (*p*=0.011). A vector containing the SNP *XPO5* rs11007 or the wild-type genotype in the 3'UTR region of *Renilla* luciferase gene was transfected in two myeloma cell lines. A reduction by 18% and 25% in the the *Renilla* luciferase activity analysis 24 hours after transfection suggests that the presence of the polymorphisms allows the binding of new miRNAs to this sequence, resulting in a significant reduction of XPO5 protein levels.Progressive disease under bortezomib treatment was more frequently observed in the case of polymorphisms in *MIR149* (p=0.033). The development of peripheral neuropathy bortezomib-related was more frequently observed in patients with polymorphism rs3360 in *KRT81* (p=0.03). **Conclusions.** A SNP in *XPO5* was significantly associated with longer OS after bortezomib treatment in patients with relapsed MM. This gene, exportin 5, mediates pre-miRNA nuclear export. This SNP could modify the miRNA biogenesis pathway through XPO5 protein levels, with a miRNA-target disturbance due to a global impairment of mature miRNAs. Concerning to the miRNA network, a polymorphism in a keratin gene (KRT81), target of diverse miRNA clusters and relevant in the structural cytoplasm framework, has been associated with clinically significant toxic neuropathy.

0556

COMPROMISED ACTIVITY OF NUCLEAR SIRTUINS SENSITIZES BRCANESS MULTIPLE MYELOMA CELLS TO DNA DAMAGE AGENTS

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Background. Multiple myeloma (MM) is a clonal malignancy of plasma cells with a striking genetic instability. This represents a very harmful disease process fundamental to the invasion and progression of MM cells. Consequently, inhibition of DNA repair mechanisms leads to significant reduction in acquisition of new genetic changes as well as progression of MM. Mammalian sirtuins are class III NAD^+-dependent histone deacetylase emerging as innovative proteins involved in multiple pathways, including genome maintenance. **Methods.** A panel of 18 different MM cell lines, both sensitive and resistant to conventional and novel anti-myeloma drugs, was used in the study. The antitumor effect of Nicotinamide combined with chemotherapic agents was investigated by CTG assay and Annexin-V/propidium iodide staining. Mechanistic studies were performed with thymidine incorporation, Western-blotting, lentivirus-mediated shRNAs and immunofluorescence. Finally, analysis of DNA DSB repair by chromosomally integrated reporter constructs followed by cytometer analysis was carried out. **Results.** We analyzed an Affymetrix GeneChip (GSE6477) for MM primary tumors (n=162) and normal plasma cells founding that transcript levels of two nuclear sirtuins (SIRT6 and SIRT7) were significantly higher in MGUS, smoldering myeloma, MM and relapsed cases when compared to normal donors. Importantly, a protein analysis assay confirmed increased levels of both sirtuins in 18 different MM established cell lines, including those resistant to novel (ANBL6-BR) and conventional (MM.1R, LR-5, Dox40) therapeutic agents, compared to PBMCs of healthy donors. Next we evaluated the functional role of Sirt6 and 7 in MM cells by using loss of function approaches with RNAi. As SIRT6 and SIRT7 silencing reduced MM cell proliferation compared with control scrambled cells but did show only a slight induction of cytotoxicity. We also examined the effects of Nicotinamide (Nam), a pan-sirtuins inhibitor, on DNA damage response signaling triggered by conventional anti-MM agents (Melphalan, Doxorubicin and 5-AZA). Nam treatment did not appreciably affect MM cell viability, conversely Nam pretreatment impaired DNA double-strand breaks (DSBs) as well as DNA repair mechanisms triggered by conventional DNA damage agents, as documented by γH2AX and RPA phosphorylation, respectively. Consistent with these findings, Nam-pretreated cells not only were impaired in forming RAD51 foci in response to Doxorubicin and Melphalan, but also caused their hypersensitivity. Importantly, this sensitizing effect was observed also in MM cells selected for resistance to Doxorubicin (RPMI-Doxo40) or Melphalan (LR5), indicating that Nam increases chemosensitivity in both drug-sensitive and -resistant MM cells. Similarly, lentivirus-mediated shRNA interference SIRT6 and 7 depletion sensitized cells to Melphalan and Doxorubicin. Finally, by using MM cell lines containing chromosomally integrated green fluorescent protein-based reporter constructs, chemical as well as genetic approaches improved the efficiency of both HR and NHEJ. Ongoing *in vivo* experiments are assessing how the chemical susceptibility of SIRT6 and/or 7-deficient cells can be exploited therapeutically. **Conclusions.** Our study strongly points towards the existence of a link between nuclear sirtuins and DNA instability of MM cells, providing the basis for further and innovative anti-MM therapeutic approach.

Allogeneic stem cell transplantation

0557

DIFFERENT PREDICTOR ROLES OF NATURAL KILLER CELL RECEPTOR GENES AND THE MISSING SELF MODEL OR LIGAND-LIGAND MODEL ON SURVIVAL AFTER HAPLOIDENTICAL TRANSPLANTATION WITHOUT T CELLS DEPLETION IN VITRO

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Backgroud. HLA-haploidentical stem cell transplantation (SCT) is a feasible therapeutic option for advanced hematologic malignancies who lack an HLA-matched related or unrelated donor. Conflicting results have been reported about the impact of alloreactivity of natural killer (NK) cells on the outcome of haploidentical SCT to leukemia patients. **Aims.** The goal of this study was to explore the predictive roles of donor Killer-cell immunoglobulin-like receptor (KIR) genotype and the missing self model or ligand-ligand model in our myeloablative HLA-haploidentical SCT without T-cell-depletion *in vitro*. **Methods.** We studied 251 patients who received haploidentical SCT. Among of which, we have 251 data of donors' and 235 data of recipients' HLA-C and Bw4, as well as 209 data of donor's KIR genes. To apply the missing ligand model, the first step was to divide the donor-recipient pairs into 2 groups according to the number of KIR ligand in donors, ie, 3 KIR ligands ("without missing self") versus fewer than 3 ("with missing self"). Meanwhile, to apply the KIR ligand-ligand model, donors who were classified as NK alloreactive against their recipients termed KIR ligand mismatched donors throughout, possessed HLA class I KIR ligand(s) which were missing in the recipients. Furthermore, genotypes for the centromeric (Cen) and telomeric (Tel) parts of the KIR locus were assigned according to the presence or absence of one or more B haplotype defining KIR genes. Donors were assigned into with or without Tel BB or Cen BB genotype as defined previously. **Results.** Among the 251 pairs of donor-recipients, 181 and 70 recipients received SCT from "with missing self"and "without missing self"donors. Using Ligand-ligand model, 183 and 52 recipients received SCT from "KIR ligand matched"and "KIR ligand mismatched"donors. The cumulative incidence of 9-year relapse rate were higher in patients received transplantation from "with missing self"or "with KIR ligand mismatched"donors compared with those from "without missing self"(p=0.014) or "without KIR ligand mismatched"(p=0.040) donors. When combined the above predictive models together, patients were subgrouped as receiving graft from "without missing self"(best, n=70), "with missing self and without KIR ligand mismatch"(better, n=113), and "with KIR ligand mismatch"(neutral, n=52), respectively. The 9-year disease-free survival (DFS) was best predicted by the combination of missing self and KIR ligand mismatch between recipients and donors pairs (RR 1.428, p=0.028 for DFS). However, donors KIR genotype with Tel BB or Cen BB (n=28) were associated with superior survival (p=0.030). **Conclusions.** These data indicate that poor prognosis after transplantation is associated with the missing self and KIR ligand mismatch in recipients and T cell alloreaction may play the predominant role in this model. However, donor selection for Tel BB or Cen BB KIR genotype, which might associate with the NK cells alloreactivity, would lead to the superior survival in patient underwent haploidentical SCT without T cells depletion *in vitro*. Therefore, the best HLA-haploidentical donors for patients underwent T-cells-replete SCT would be with favorable Tel BB or Cen BB KIR genotype and without missing self between donor and recipients

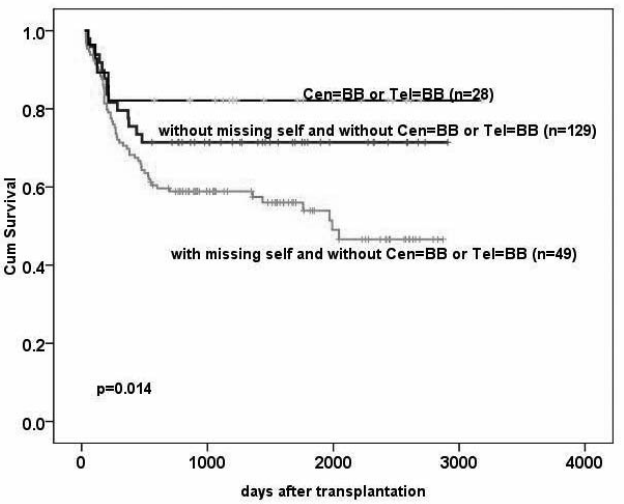

Figure 1. Disease free survival.

0558

ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) FOR CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML): A REPORT FROM THE SOCIETE FRANCAISE DE GREFFE DE MOELLE ET DE THERAPIE CELLULAIRE (SFGM-TC)

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Background. CMML is a heterogeneous disease with overall survival (OS) ranging from 12 mo to several years, where few series of allo-SCT have been published. This retrospective study aimed at determining prognostic factors for OS after allo-SCT in a group of consecutive 73 CMML patients reported to the SFGM-TC registry between 1992 and 2009. **Methods.** For this analysis, in addition to classical demographic and transplant characteristics, other characteristics at diagnosis and at transplant, including WHO classification in CMML 1 and 2, IPSS in patients with WBC <13G/L, and prognostic factors published by the GFM in CMML with WBC >13G/l, interval between diagnosis and allo-SCT, and prior treatments were analyzed. **Results.** at diagnosis M/F was 49/24, median age 53 yrs (range, 27-66). 36% patients had palpable SPM, 70% WBC>13X10^9/L. 48/13/9 patients had good/int/poor risk karyotype according to IPSS, 61% patients had CMML1, and 39% CMML-2. Of the 22 patients with WBC<13G/l, six had int-2 and 1 had high risk IPSS, while of the 45 patients with WBC>13G/l, 37 had at least 2 of our published poor prognostic factors. Before allo-SCT, 26 patients had received intensive anthracycline-cytarabine chemotherapy (CT), 21 low dose CT (18 HY, 3 VP16) and, 6 hypomethylating agents. 32 patients (44%) developed infection (bacterial or fungal) between diagnosis and allo-SCT.Median interval from diagnosis to allo-SCT was 10.6 mo (range 2.8-80). At time of allo-SCT, 15 patients had responded to AML like therapy, while 42 patients were treatment failure or in relapse, or had not been treated, including5 AML progressions. Nineteen patients still had palpable SPM before allo-SCT. The donor was an HLA-identical, unrelated and haploidentical sibling in 41/31/1 cases respectively. 43 patients (59%) received reduced-intensity conditioning (RIC), while the other received MAC. With a median follow-up of 23 mo (1-145), grade 0-1 acute GVHD and grade 2-4 developed in 23 and 21 patients respectively. Chronic GVHD was present in 25 patients (35%) (limited:15, extensive: 10; cum incidence: 37%at 3yrs).The 2- and 3-yr OS were 42% and 32%, respectively. The 3-yr cum incidence of NRM was 36%. The 3-yr relapse-free survival was 30%. OS was not influenced by the disease status at allo-SCT, including CMML1 vs CMML2, IPSS score (WBC<13G/l) and GFM score (WBC>13G/L), the number of prior treatments, HLA matching and cGVHD. However, palpable SPM at transplantation was a negative prognostic factor for OS (2-yr OS: 52% vs.28%, p=0.03). Presence of palpable SPM was correlated with thrombocytopenia, presence of peripheral blasts and myelemia. 2-yr DFS was better after 2004, year of transplantation (57% vs 19%, p=0,016). In multivariate analysis, the strongest prognostic factor for OS was palpable SPM at transplantation (HR=0,48 95%CI:0.24-0.98; p=0.042). **Conclusions.** Allo-SCT is a valid treatment option for CMML patients eligible to this treatment and is improving. Palpable SPM at transplantation was the only independent negative prognostic factor. Most patients were however treated before the advent of hypomethylating agents. The use of RIC regimens combined to those agents (and possibly to other novel agents) prior and after allo-SCT may further improve outcome.

0559

ALLOGRAFTING FROM UNRELATED DONORS IN MULTIPLE MYELOMA: A STUDY BY THE ITALIAN BONE MARROW DONOR REGISTRY

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Background. The role of allografting in multiple myeloma is controversial **Aims.** To evaluate clinical outcomes of allografting from unrelated donors in the treatment of myeloma **Methods.** We conducted a retrospective study from 2000 through 2009 through the Italian Bone Marrow Donor Registry.

Table 1.

Conditioning	Myeloablative	Reduced-intensity	Non-myeloablative
Patient number (%)	52/185 (28)	69/185 (37)	64/185 (35)
Median Age	45	53	55
Previous therapy lines < 2 (%)	23 (27)	33 (38)	30 (35)
Previous therapy lines ≥ 2 (%)	29 (29)	36 (37)	34 (34)
Stem Cell Source BM (%)	24 (57)	18 (43)	0 (0)
Stem Cell Source PBSC (%)	28 (19)	51 (36)	64 (45)

Results. Overall, 196 myeloma patients, median age 51 years (32-67), were transplanted at 34 Centers. Fifty-two (28.1%), 69 (37.3%), and 64 (34.6%) patients were treated with myeloablative, reduced-intensity and non-myeloablative conditioning respectively.Incidence of acute grade II-IV graft-versus-host-disease (GVHD) was 46.4% whereas chronic GVHD was 45.1%. There was no difference in GVHD incidence among the 3 cohorts. Complete and partial remissions in patients who survived at least 3 months post-transplant were 27% and 28%. At a median follow up of 32 (0-118) months post-transplant, in the entire study population, median OS from diagnosis was 70.6 months while OS and EFS from the allograft were 18.9 and 14.9 months. Overall, the cumulative incidence of transplant related mortality (TRM) was 29.6% at 1 year and 32.4% at 5 years. OS from diagnosis and EFS from transplant were 70.6 and 28.2 months; 66.8 and 9.1 months; 111.9 and 22.4 months in patients who respectively underwent a myeloablative, a reduced-intensity and a non-myeloablative transplant. One-year and 5-year TRM was 33.3% and 35.7%, 32.2% and 34.4%, and 22.1% and 26.5% respectively. By univariate analysis, lower number of chemotherapy lines before the allograft, disease status at transplant, a fully HLA-identical donor, the use of peripheral hematopoietic cells

rather than bone marrow were statistically significant variables for better OS whereas disease status at transplant, a fully HLA-identical donor, chronic GVHD (either limited or extensive) were statistically significant for better EFS. However, by multivariate analysis, only the development of chronic GVHD (HR 0.50; p<0.001) and a better response post-transplant (HR 2.11; p<0.03) were significantly associated with longer OS whereas chronic GVHD was the only variable associated with better EFS (HR 0.32; p<0.001). Acute GVHD was associated with both poorer OS (HR 2.35; p<0.001)) and EFS (HR 3.19; p<0.001). **Conclusions.** There appears to be a strong association between chronic GVHD and graft-vs.-myeloma effects. However, long term disease control remains an issue regardless of the conditioning employed. Prospective trials may allow to define which patient category may most benefit from an unrelated donor allograft.

0560

DISTINCT RECEPTOR PROFILE DEFINES ALLOREACTIVE T CELLS BEFORE GVHD ONSET

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Background. Acute graft-versus-host disease (aGVHD) poses a major complication after allogeneic hematopoietic cell transplantation (allo-HCT). This immune syndrome is mediated by alloreactive donor T cells that attack the gastrointestinal tract, liver and skin. Previously, we and others demonstrated that alloreactive T cells are primed in secondary lymphoid organs. Therefore, we reasoned that in order to cause aGVHD alloreactive T cells require to express appropriate homing receptors to efficiently migrate from their priming sites to the gastrointestinal tract, liver and skin. **Aims.** To identify alloreactive T cells in the peripheral blood (PB) by a panel of homing receptors before clinical aGVHD manifestation. **Methods.** In a preclinical minor antigen mismatch (miHAg) allo-HCT mouse model we transplanted luciferase-expressing C57Bl/6 (H-2b, Thy1.1) T cells plus C57Bl/6 wild type bone marrow (Thy1.2) into conditioned (8Gy) Balb/B (H-2b, Thy1.2) recipients. MHC major mismatched Balb/C (H-2d, Thy1.2, 8Gy) and syngeneic C57Bl/6 WT (H-2b, Thy1.2, 9Gy) recipients served as controls. PB donor T cells were characterized by FACS at previously identified peak time points of T cell migration (days+4, +11, +15). Histopathological scorings confirmed GVHD. **Results.** Clinically apparent aGVHD developed by day+21 after miHAg mismatched allo-HCT. When we analyzed the peripheral blood of miHAg allo-HCT recipients we found CC chemokine receptors CCR5 and CCR9 highly upregulated (47% and 70% on day+4) in comparison to untreated C57Bl/6 control mice (<10% of PB CD8+ T cells). Mean fluorescence intensity (MFI) of CCR5 on day+15 of CD8+ donor T cells in the allogeneic setting exceeded that of syngeneic controls by 2-fold.Importantly, alloreactive donor T cells highly upregulated α4β7 integrin after miHAg mismatch allo-HCT. Relative expression values (26% CD4+α4β7+; 60% CD8+α4β7+) and absolute cell numbers (3.4x10^4 CD4+α4β7+ cells/ml; 15.8x10^4 CD8+α4β7+ cells/ml) exceeded α4β7-expressing T cells in syngeneic controls (20% CD4+α4β7+; 5% CD8+α4β7+; 0.9x10^4 CD4+α4β7+ cells/ml; 0.3x10^4 CD8+α4β7+ cells/ml) on day+11. Interestingly, most allogeneic T cells were α4β7+αEβ7- in contrast to double positive α4β7+αEβ7+ syngeneic T cells. Furthermore, CD8+ donor T cells highly upregulated P-selectin-ligand (35% CD8+P-selectin-ligand+; 9.1x10^4 cells/ml) and E-selectin-ligand (21% CD8+E-selectin-ligand+; 2.7x10^4 cells/ml) in contrast to syngeneic controls (10%, 0.7x10^4 and 9%, 0.2x10^4 cells/ml, respectively). Activation markers such as CD44 were expressed on 80-100% PB T cells in either syngeneic or allogeneic recipients.Conclusion:Our data indicates that alloreactive T cells can be identified early in the peripheral blood upon their homing receptor expression profile before the onset of aGVHD. Relative and absolute expression levels of α4β7 integrin, αEβ7 integrin, P- and E-selectin-ligand homing receptors and MFI values of CCR5 but not CCR9 on CD4+ and CD8+ T cells identified alloreactive T cells 6 to 10 days before the onset of clinically apparent aGVHD symptoms. Our data suggests these markers as potential predictors for aGVHD.

0561

T-CELLS SPECIFIC FOR THE ASPERGILLUS PROTEINS CRF1 AND CATALASE1 DEVELOP IN PATIENTS RECOVERING FROM INVASIVE ASPERGILLOSIS

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Background. Invasive aspergillosis is a common and life-threatening complication in recipients of allogeneic stem cell transplantation (SCT). Patients are most severely at risk in the neutropenic phase, but there is increasing evidence that impaired T-cell mediated immunity also increases the risk of invasive aspergillosis. In healthy individuals we identified *Aspergillus*-specific T-cells by measuring CD154 expression and IFNγ production in response to stimulation with overlapping peptides of the *A. fumigatus* proteins Crf1 and Catalase1. Antigen specific CD4+ T-cells were single cell sorted to identify the recognized epitopes and *Aspergillus* specificity was confirmed, based on reactivity of the different T cell clones to dendritic cells loaded with *Aspergillus* crude extract or *Aspergillus* recombinant protein. **Aims.** To allow development of new therapeutic strategies including adoptive transfer of antigen specific T-cells, we investigated the role of *Aspergillus*-specific T-cells in the clearance of aspergillus infection, and characterized the T-cell mediated immune responses against *A. fumigatus* in patients with invasive aspergillosis. **Methods.** We analyzed the T-cell response against *A. fumigatus* in 7 patients after allogeneic SCT who were diagnosed with probable or proven invasive aspergillosis. All patients had received an allogeneic SCT because of a hematological malignancy and aspergillus infection was diagnosed 1 to 10 months after SCT. Peripheral blood mononuclear cells (PBMC) were stimulated with the overlapping peptides of Crf1 and Catalase1 at several time points before and after the diagnosis of invasive aspergillosis and analyzed by flowcytometry using intracellular staining for IFNγ and CD154. **Results.** Directly ex vivo no *Aspergillus*-specific T-cells could be detected by intracellular staining for IFNγ and CD154. However, when PBMC were stimulated with the peptide mixtures of Crf1 and Catalase1, cultured for 7 days in the presence of IL-2 and restimulated with Crf1 and Catalase1 we detected clear populations of *Aspergillus*-specific T-cells in 6 out of 6 patients with regression of aspergillus lesions or stable disease.In these 6 patients a peak of Crf1- and Catalase1-specific CD154 expressing CD4+ T-cells was present around the time of regression of aspergillus lesions. The peak frequency of Crf1- and Catalase1-specific CD154 expressing T-cells varied between 0.5 and 3% of CD4+ T-cells. In most patients both Crf1- and Catalase1-specific T-cells were present. After clearance of the aspergillus infection the frequency of *Aspergillus*-specific CD4+ T-cells decreased to low or undetectable levels. In one patient no Crf1- or Catalase1-specific T-cells could be identified. This patient had progressive aspergillus infection while suffering from severe GvHD of the skin, liver and colon and died shortly after the diagnosis of invasive aspergillosis. **Conclusions.** Using only 2 *A. fumigatus* proteins as target antigens, we demonstrated the induction of *Aspergillus*-specific T-cells in 6 patients, coinciding with the decline of aspergillus lesions. These data indicate that an immune response directed against *A. fumigatus* proteins helps to clear an aspergillus infection. Therefore, *Aspergillus*-specific T cells generated *in vitro* by stimulating with *A. fumigatus* proteins like Crf1 and Catalase1, may be used for adoptive T-cell therapy for invasive aspergillosis.

Bleeding disorders

0562

STOP CODON READTHROUGH WITH PTC-124 AND GENTAMYCIN IN HEMOPHILIA A CAUSED BY NONSENSE MUTATIONS

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Background. The hemophilia A is caused by the mutations in the factor Ⅷ gene. One of the most important molecular mechanisms of hemophilia A is nonsense mutation,which lead to the production of nonfunctional truncated, nonfunctional or deleterious protein. Aminoglycoside antibiotics, such as Gentamycin, could induce readthrough of premature termination codon (PTC) and increase the production of the protein with nonsense mutation. However, repeated use of high doses of aminoglycosides for the readthrough therapy, has significant adverse effects including the nephrotoxicity and ototoxicity. PTC-124, a novel and non-toxic chemical compound, could be able to accomplish the task. **Aims.** At present, due to lacking of the eradicative therapy, the hemophilia A patients should infusion the expensive blood coagulation factor in their lifetime. So searching for the new drugs has significant social effects. Through our study, we want to compare the readthrough ability of different concentrations of PTC-124 and Gentamycin in hemophilia A caused by different PTC and provide the basis for the further researches of readthrough therapy. **Methods.** The international hemophilia mutation database was analyzed for finding the hot spots of nonsense mutation sites in FⅧ gene. The nonsense mutants were constructed by site-directed mutagenesis. Detecting the HEK-293 cells viability after treated with different concentrations of PTC-124 and Gentamycin for 48h, using CCK-8(Cell Counting Kit-8). Then, HEK-293 cells were transfected with either the wild-type or mutated constructs by liposome-mediated gene transfer method, then treated with different concentrations of PTC-124 and Gentamycin for 48h. The mRNA expression levels of FⅧ gene were detected by real-time PCR. The procoagulant activity (FⅧ：C) and antigen (FⅧ：Ag) of FⅧ in the cell lysate were detected using one stage method and ELISA, respectively. **Results.** The hot spots of nonsense mutation sites and our objects in FⅧ gene were W14X R1696X K1827X and R2307X. Except the designed sites,there was no other nucleotide mutations in the sequences of four mutants. The IC50 of PTC124 and Gentamycin in HEK-293 cells were 111.585±9.597μM and 4.23±0.32mM respectively, then select different concentrations of PTC124 and Gentamycin for the readthrough study. Compared with the untreated controls ,the mRNA expression levels of FⅧ gene and the procoagulant activity (FⅧ：C) and antigen (FⅧ：Ag) of FⅧ in the cell lysate treated with different concentration of PTC124 and Gentamycin were significantly elevated (P□0.05) in those four mutants.**Conclusion** From our data, PTC-124 had the similar readthrough activity for those four mutants in FⅧ gene compared with Gentamycin, while with less concentration and toxicity. In hemohilia A, even a small increase in FⅧ expression level can dramatically ameliorate the clinical phenotype, so the readtrhough approach can be a good therapeutic strategy. Although the aminoglycoside antibiotics, such as Gentamycin, could increase the functional protein prodution, however, these drugs have serious side effects at therapeutically relevant concentrations. PTC124, which is not structurally related to aminoglycosides, could be able to provide a better treatment for hemophilia A caused by nonsense mutation.

0563

INTRACRANIAL HEMORRHAGE IN PATIENTS WITH A HEMATOLOGICAL DISORDER REQUIRING INTENSIVE CHEMOTHERAPY AND/OR A STEM CELL TRANSPLANT: A SYSTEMATIC REVIEW

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Background. To advance the quality of care for hematology patients receiving intensive chemotherapy it is important to gain a greater understanding of the risk factors for life-threatening haemorrhage. This systematic review concentrates on intra-cranial hemorrhage (ICH) because it is the most serious type of bleed caused by significant thrombocytopenia. If an ICH does not cause death it may lead to significant long-term morbidity. However, this complication is rare, its exact incidence is uncertain, and predisposing risk factors are currently unknown. **Aims.** A systematic review of cases published in the literature during the last decade to determine its incidence and possible risk factors. **Methods**. We searched MEDLINE, EMBASE, CENTRAL, NLH CINAHL, and the SRI RCT Handsearch(www.transfusionevidencelibrary.com) electronic bibliographic databases for studies published between January 2001 and December 2010. There were no language restrictions. We included studies of patients, of any age, with hematological disorders, who required intensive chemotherapy or a stem cell transplant, and were expected to develop a significant thrombocytopenia. If trials consisted of mixed populations of patients, with diagnoses of solid tumours, only data from the haematological sub-groups were used. We excluded studies if fewer than 80% of participants had a hematological disorder and sub-group data was not provided. We also excluded studies if the participants were receiving palliative treatment; fewer than 33% of participants were expected to become significantly thrombocytopenic, or participants had immune thrombocytopenia. Data were collected on baseline characteristics of the cases (age, diagnosis, treatment and presumed risk-factors for ICH(e.g sepsis, anticoagulants, coagulopathy)) as well as any data on the same characteristics that were reported for the entire study population. **Results.** 6852 studies were initially identified, 6002 studies were excluded on the basis of the abstract. 850 full text articles were reviewed and of these, 254 studies were included in the review (183 cohort studies, 22 case series, and 49 case studies). 1099 cases of ICH were described within the included studies. Over half(55%) died due to ICH. The majority of cases were poorly described, for example, only 26.7%(294/1099) reported whether the patient was thrombocytopenic, 18.1% whether patient had a coagulopathy and only 4.9% described the presence or absence of anticoagulants. The majority of patients (93.4%;511/547) whose disease status was known had active disease at the time of the ICH. Data on incidence ratios will be presented at the meeting. Studies which included patients with acute promyelocytic leukemia (APL), which is well known to have a high incidence of hemorrhage, were more likely to report the presence or absence of ICH. Overall, approximately a third of acute leukemia patients within the included studies had APL, whereas the actual incidence of APL is approximately 10%. **Summary and Conclusions.** Cases of ICH are poorly reported in the literature, and the literature is biased in favor of APL. To provide a more unbiased picture of the incidence and risk-factors for ICH in hematology patients we have set up a UK-wide prospective reporting system (Haematology Active Surveillance System (HASS)) with a case-control study (InCiTe study) embedded within it.

0564

GLA-DOMAINLESS ACTIVATED FACTOR X: A MOLECULAR BAIT TO BYPASS A BLOCKED TENASE COMPLEX

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Background. Hemophilia is caused by deficiencies in coagulation factor VIII or IX, resulting in a direct blockade of the intrinsic tenase complex and an indirect one of the extrinsic tenase complex that is rapidly inhibited upon binding of factor Xa to Tissue Factor Pathway Inhibitor. Therefore, we evaluated the ability of a truncated form of factor Xa devoid of procoagulant properties, Gla-domainless factor Xa, to bind to Tissue Factor Pathway Inhibitor and to alleviate the physiological inhibition of the extrinsic tenase. **Materials and Methods.** Using thrombin generation assay triggered by a low concentration of tissue factor, we evaluated the ability of Gla-domainless factor Xa to restore blood coagulation in plasma from hemophilia A and B patients without and with inhibitors. We then compared its efficacy to generate thrombin to depletion of antithrombin or Tissue Factor Pathway Inhibitor by specific antibodies. Finally, we compared the kinetics of neutralization of factor Xa and Gla-domainless factor Xa by antithrombin and Tissue Factor Pathway Inhibitor.Results. Gla-domainless factor Xa was able to restore thrombin generation in hemophilia plasmas. This effect was observed for plasmas from hemophilia A, without or with inhibitors, and hemophilia B donors. Gla-domainless factor Xa had a lower affinity for Tissue Factor Pathway Inhibitor than factor Xa whereas the affinities of both proteins to antithrombin were similar. Finaly, despite a short half-life in plasma, the effect of Gla-domainless factor Xa on thrombin generation was sustained for at least one hour.Conclusion. As Gla-domainless factor Xa was able to restore thrombin generation in plasma from hemophilia patients, our results suggest that it may be an effective alternative to current treatments for hemophilia with or without an inhibitor.

0565

SYNOVIORTHESIS BY RIFAMPICIN IN PEDIATRIC PATIENTS WITH CHRONIC HAEMOPHILIC SYNOVITIS : A SINGLE PROSPECTIVE CENTER STUDY

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Background. Intra-articular bleeding (haemarthrosis) is the most common manifestation of haemophilia A (HA) and haemophilia B (HB) producing hypertrophic synovitis and, in the long term, progressive cartilage degradation leading to irreversible arthropathy. Rifampicin has demonstrated proteolytic and antifibrinolytic actions, producing fibrosis and sclerosis of the hypertrophic synovium when injected into the joints. **Objective**. The aim of the study is to assess clinical outcomes and standard scores in 23 patients with hemophilic arthropathy undergoing chemical synovectomy with rifampicin. **Methods.** Twenty-three Hemophiliacs with target joints were enrolled and followed-up prospectively; all were inhibitor negative; 13 severe HA, 8 moderate HA and two severe HB in the period between January 2011 and January 2012. The indication for synoviorthesis was chronic synovitis characterized by recurrent haemarthrosis, persistent pain and limited range of motion (ROM). All patients were on-demand replacement therapy. Their median age was 12 years (range 5- 18 years) .A schedule was adopted to treat each joint with 5 doses of intra-articular rifampicin once a week . The dose used for knees was rifampicin(500 mg) injected intra-articularly with 4 mL lidocaine 2% and for elbows and ankles, the dose was 250 mg, with 2 mL lidocaine 2% once a week. Patients were covered with 3 doses of factor FVIII 25 U/Kg; first dose one hour before injection with good FVIII recovery followed by two same doses every12 hours . Oral analgesia was offered as required because of acute but transient painful inflammatory reaction and compression bandage was applied. World Federation of Hemophilia score was assessed 24 hours before intra-articular injection and one month later; score improvement was considered from excellent to fair according to evaluation. Physiotherapy was initiated as early as could be tolerated. **Results**. The procedures were performed on twenty nine joints(17 knees, 8ankles, 4 elbows) with a total of 145 injections . After the procedures were done : 19 (82%) were considered effective injections (as excellent or good), while four(18%) were considered insufficient (fair or poor). Pain was reduced in 79% of cases and bleeding episodes were reduced from 2.8 to 1.4 per joint / month before and after the chemical treatment respectively . The mean (WFH) score was 11.5 (range 5-21) before synoviorthesis and 6 (range 3- 12) after treatment. Non had hypersensitivity reaction to rifampicin. **Conclusions.** Intra-articular injection with rifampicin appears to be effective in reducing joint pain and in improving the ROM that can be given to many patients simultaneously on an outpatient basis. This simple procedure presents a low risk of bleeding and a low cost of therapy by reducing haemarthrosis effectively . Its especially practical in developing countries where radioactive isotopes are not easily available.

0566

UPREGULATION OF ANNEXIN II BY PML/RARALPHA FUSION PROTEIN ENHANCES FIBRINOLYSIS AND CELL INVASION IN U937 CELLS

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Background. Life-threatening coagulopathy usually occurs in acute promyelocytic leukemia (APL), mainly due to hyperfibrinolysis, which is triggered by annexin II, a cofactor for plasminogen and t-PA to accelerate plasmin generation. High level of annexin II in APL blast cells leads to excessive production of plasmin, resulting in massive degradation of fibrinogen and fibrin, and destroying extracellular matrix. While APL have achieved high rate of complete remission, 5%-13.3% patients experience early death and 5% relapse with central nervous system leukemia. This indicates that coagulopathy and infiltration have emerged as challenges for further improving treatment efficacy. PML/RARalpha fusion protein is specific for APL and serves as the target of differentiation therapy, therefore we hypothesize aberrant expression of annexin II may be associated with PML/RARalpha fusion protein. **Aims.** To verify the hypothesis on regulatory expression of annexin II by PML/RARalpha fusion protein, and to investigate the role of aberrant expression of annexin II in fibrinolysis and cell invasion. **Methods.** U937/PR9 cells, stably expressing PML/RARalpha fusion protein in the presence of 100 μmol $ZnSO_4$, were used to explore the association between annexin II and PML/RARalpha protein at both transcriptional and translational levels by RT-PCR and western blot. U937/MT cells contain only eukaryotic vector as control. The effects of annexin II on fibrinolysis and leukemic cell invasion were studied using plasminogen generation assay and matrix invasion assay. **Results.** PML/RARalpha fusion protein was detected 1 hour after the addition of 100 μmol $ZnSO_4$, followed by marked increase of annexin II protein at 2 hours with lagging time of 1 hour (Figure A). In addition, RT-PCR assay showed that increased mRNA expression of annexin II after addition of zinc as against without zinc (Figure B). Expression of annexin II remained stable in U937/MT cells with addition of 100 μmol $ZnSO_4$, indicating $ZnSO_4$ had no effect on expression of annexin II (Figure C). To investigate the functional significance of increased annexin II expression, we examined fibrinolysis and cell invasion in U937/PR9 cells in presence or absence of zinc. The data demonstrated that the initial rate of plasmin generation speeded up to 4.2 times with addition of zinc, and was abolished to constitutive capacity when the cells were preincubated with monoclonal anti-annexin II antibody. In matrix invasion assay, high level of annexin II promoted cell invasion rate to 80% (with zinc) versus 43% (without zinc), and this elevated invasion rate was also diminished by preincubation with monoclonal anti-annexin II antibody (Figure D). **Conclusions.** The current finding raveled that PML/RARalpha fusion protein facilitates annexin II expression, which in turn mediates fibrinolysis and cell invasion in U937/PR9 cells. Annexin II may be a potential target for the optimization of APL treatment.

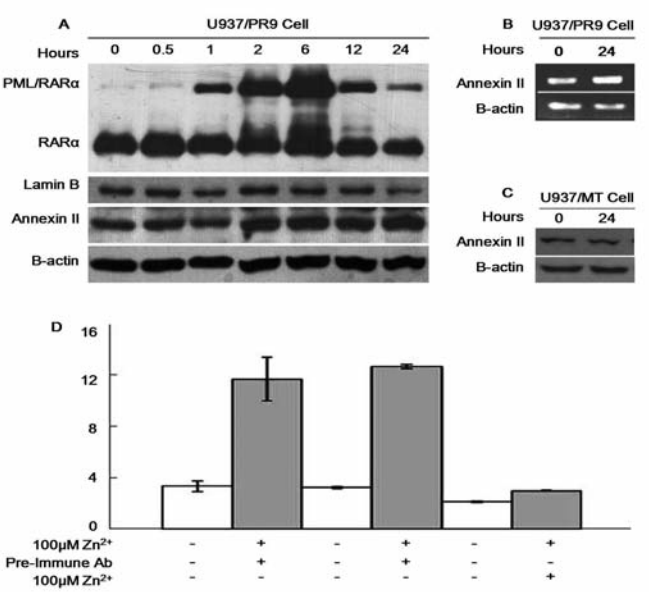

Figure 1.

Presidential Symposium

0567

EXOME SEQUENCING IDENTIFIES MUTATION OF THE RIBOSOME IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. T-cell acute lymphoblastic leukemia (T-ALL) is caused by accumulation of somatic mutations in developing T-cells. Major driver mutations in T-ALL include hyperactivity of the NOTCH1 pathway, activation of JAK/STAT signaling, overexpression of a set of transcription factors, and loss of cell cycle control. Although pediatric T-ALL patients respond well to the current chemotherapeutic regiments, the treatment is very toxic and the survival of older patients is still below 40%.**Aims.** To gain insight in the spectrum of mutations present in T-ALL and to identify potential new targets for therapy, we performed exome sequencing in both pediatric and adult patients. **Methods.** Whole exome sequencing was performed on 36 paired diagnosis-remission T-ALL samples, 18 diagnosis only samples and on 17 T-ALL cell lines. **Results.** We initially limited our analysis to the 36 paired sample sets for discovery of novel somatic mutations. On average, we identified 13 somatic protein-altering mutations (single nucleotide variations and small insertions and deletions) per sample. 389 genes were mutated in this samples set, of which 35 genes were selected as candidate drivers because they showed a significantly higher mutation rate compared to the local background mutation rate (24/389 genes) or because they were recurrently mutated in at least 2 of the 36 samples (31/389 genes). Interestingly, the 35 selected genes contained an overrepresentation (8/35 genes) of genes on the X-chromosome, which may be linked to the male predominance in T-ALL. Ten of the selected genes (*PHF6*, *FBXW7, PTEN, WT1, NOTCH1, DNM2, JAK3, BCL11B, MYB*, and *EZH2)* are known oncogenes or tumor suppressors in T-ALL. The remaining 25 genes are potential novel oncogenic drivers in T-ALL, with functions in signal transduction (*TLR1, LPHN2, USP9X*), transcriptional regulation (*CNOT3, ZNF711, TBP*) and epigenetic regulation (*TET1, KDM6A, TDRD6*). Strikingly, 7 of 54 T-ALL cases (13%) harbored mutations in RPL5 or RPL10, two proteins that are part of the ribosome, the multi-protein complex that translates mRNA into proteins in the cell. Mutation of Arginine 98 in the X-linked *RPL10* gene was identified in 8.4% of pediatric T-ALL cases. By performing functional studies in yeast, we could document a clear ribosome biogenesis defect in cells expressing the Rpl10 R98S mutant. While more experiments are needed to determine the exact consequences of the ribosomal protein mutations in T-ALL, the yeast results suggest that ribosome mutations may cripple the ribosome in T-ALL. **Summary and Conclusions.** Our data provide insights in the landscape of mutations in T-ALL and identify the ribosome as a novel oncogenic factor and potential target for therapy in T-ALL.

0568

MULTIPLE INHIBITORY LIGANDS INDUCE T CELL IMMUNOLOGICAL SYNAPSE DYSFUNCTION IN HEMATOLOGIC MALIGNANCY THAT CAN BE BLOCKED WITH LENALIDOMIDE - DEFINING A REVERSIBLE IMMUNE EVASION MECHANISM IN CANCER

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Background. Cancer cell immune evasion is an emerging hallmark of disease progression. We have previously demonstrated that impaired actin polymerization at the T cell immunological synapse is a global immunosuppressive mechanism in chronic lymphocytic leukemia (CLL). Direct contact with tumor cells induces defective actin polarization at the immune synapse in previously healthy T cells. **Aims.** Identify the molecules mediating T cell immune synapse dysfunction in CLL. **Methods.** We designed a small interfering RNA (siRNA) functional synapse bioassay. This used the B cell leukemia cell line MEC1 (that induces the same T cell immune synapse defect as primary CLL cells) and customized siRNA libraries that included soluble and membrane immunosuppressive molecules.siRNA treated MEC1 cells were co-cultured with healthy donor T cells which were subsequently negatively selected and used in conjugation assays with superantigen (sAg)-pulsed third-party B cells as antigen-presenting cells (APCs). T cell F-actin polymerization was quantified using confocal image analysis software (Axiovision and Cellomics, Zeiss). In this bioassay, knockdown of tumor inhibitory molecules should lead to gain in T cell synapse function. **Results.** We identified three B7-related molecules CD200, CD274 (PD-L1), and CD276 (B7-H3), and one TNF-receptor superfamily member CD270 (TNFRSF14 or HVEM), for which siRNA treatment of MEC1 cells significantly enhanced immune synapse formation. We confirmed these results using primary CLL cells pre-treated with neutralizing antibodies and show that the combined action of these molecules are co-opted by CLL cells to induce potent immunosuppressive effects on both autologous and allogeneic T cell actin dynamics and effector function. In contrast, no effect on T cell synapse function was seen using healthy B cells treated with or without neutralizing antibody. Next, *in situ* immunohistochemistry analysis showed that all four inhibitory ligands were up-regulated in CLL compared to reactive lymph node tissue and linked to poor prognosis. We further show that impairment of actin dynamics in T cells is a common immunosuppressive strategy used by both hematologic (including follicular lymphoma and diffuse large B cell lymphoma) and solid carcinoma cells (including ovarian cancer and squamous cell carcinoma) and mediated by the activity of these inhibitory ligands. We demonstrate that this immunosuppressive signaling targets T cell Rho GTPase activation. Co-culture with CLL cells, compared with healthy B cell control cells, significantly decreased activated RhoA, Rac1 and Cdc42 levels in T cell receptor-stimulated cells. Of clinical relevance, the immunomodulatory drug lenalidomide prevented induction of these defects *in vitro* and *in vivo* (clinical trial samples) and mimicked the effect of antibody blockade by down-regulating tumor cell inhibitory ligands and inhibitory receptors on T cells during immunosuppressive co-culture interactions. **Conclusions.** These results using human CLL as a model cancer establish a novel evasion mechanism whereby malignant cells exploit multiple inhibitory ligand signaling to down-regulate small GTPases and lytic synapse function in global T cell populations. These findings should contribute to the design of immunotherapeutic strategies to reverse T cell tolerance in cancer.

0569

DIRECT REGULATION OF THE GFI1 GENE BY AML1-ETO CONTRIBUTES TO LEUKEMIA DEVELOPMENT

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Background and Aims. In over 10% of patients with acute myeloid leukaemia (AML) the translocation t(8;21) is present. This translocation results in the formation of an AML1-ETO oncofusion protein. AML1 is a subunit of the transcription factor CBF and ETO is a transcriptional co-repressor. The oncofusion protein contains the DNA binding domain of AML1 and almost the entire coding region of ETO. AML1-ETO contributes to leukaemia development through altered gene expression. Despite this knowledge, the exact molecular function of AML1-ETO is still poorly understood. Also genes directly deregulated by AML1-ETO implicated in disease development remain largely unknown. **Methods and results.** To gain insight into the molecular function of AML1-ETO, we performed genome-wide ChIP-seq experiments to identify genes directly bound

by AML1-ETO. We observed that AML1-ETO interacts with DNA sequences of the core promoter of the oncogene *GFI1* in primary leukemic cells. GFI1 is a transcription factor that represses gene expression. One of the genes normally silenced by GFI1 is the *GFI* gene itself. The fact that AML1-ETO binds to the *GFI1* gene is important because we reported recently that AML1-ETO also directly interacts with the GFI1 protein through the ETO moiety. In addition, we showed that AML1-ETO inhibited the auto-repressive function of GFI in reporter assays. Here we studied the relevance of this finding by analyzing *GFI1* RNA expression in a cohort of over 500 AML cases. This revealed that *GFI1* gene expression was significantly higher in AML1-ETO-positive leukaemia compared to other leukaemia subtypes (including the inv(16)/CBFB-MYH11) and to normal bone marrow stem cells (p<0.001). In AML1-ETO positive leukaemia, the *GFI1* paralogue *GFI1B* was not highly expressed and AML1-ETO did not interact with the *GFI1B* gene. Thus, *GFI1* is specifically induced in AML1-ETO positive leukaemia. Subsequent biological studies using lentivirally-mediated gene silencing showed that GFI1 is required for growth of AML1-ETO positive Kasumi1 cells. We also studied the role of GFI1 in leukaemia development *in vivo* by crossing conditional *AML1-ETO* knockin mice with *GFI1* null mice. Poly-IC induced CRE expression resulting in AML1-ETO expression followed by G-CSF and ENU treatment resulted in leukaemia development in over 80% of *AML1-ETO/GFI1* wild type mice within one year. In sharp contrast, none of the *AML1-ETO/GFI1* null mice developed leukaemia (n = 21 for both conditions, p<0.02). This indicates that GFI1 is required for AML1-ETO induced leukemia in mice.**Summary and Conclusions.** AML1-ETO binds the *GFI1* gene and inhibits the auto-repressive function of the GFI1 protein. This results in high *GFI1* expression in AML1-ETO positive leukemia. AML1-ETO also interacts with and needs the GFI1 protein for malignant transformation. Because GFI1 interacts with ETO, genes normally regulated by GFI1 could be de-regulated through the interaction with AML1-ETO. Vice versa, AML1-ETO may deregulate AML1 target genes through recruitment of the repressor GFI1. GFI1 may represent an important therapeutic target in AML1-ETO positive leukaemia.

0570

BONE MARROW FAILURE IN FANCONI ANEMIA IS TRIGGERED BY AN EXACERBATED P53/P21 DNA DAMAGE RESPONSE THAT IMPAIRS HEMATOPOIETIC STEM CELLS

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Background. Fanconi anemia (FA) is due to inherited mutations of one of the 15 *FANC* genes involved in the FA/BRCA DNA repair pathway. In addition to congenital abnormalities, FA patients undergo progressive bone marrow failure (BMF) during childhood and they have a strong susceptibility to MDS/AML. **Aims.** The mechanisms underlying BMF have been elusive to date, largely because of practical difficulties in a disorder with poor bone marrow (BM) cells, and because murine Fanc$^{-/-}$ models do not fully recapitulate the BMF phenotype. In order to investigate BMF pathogenesis in FA, we conducted a study based on a large cohort of FA patients, and modeled the findings in mouse systems.**Methods.** Fresh BM cells from 91 FA patients were collected during BM aspirate and proceeded for CD34$^+$ purification and CFU-GM assays. Two *in vivo* models of hematopoietic transplant of FA cells were developed using *Fancg-/-* mice and human CD34$^+$ cells. Moreover, we generated double knockout *Fancd2-/-p53-/-* mice and analyzed hematopoietic stem and progenitor cells (HSPCs). **Results.** CD34$^+$ cell counts and *in vitro* clonogenic activity were lower in FA patients compared to 40 healthy BM donors (P<10^{-15}). Interestingly, even the youngest FA patients investigated before the onset of BMF had lower values, suggesting that the HSPCs impairment began before birth.As FA cells have a constitutional DNA repair defect, we investigated the cellular response to DNA damage. FA HSPCs had a strong activation of p53 and p21, leading to a delayed G0/G1 cell-cycle arrest after resolution of an early G2 checkpoint. Moreover, shRNA-mediated knockdown of p53 or p21 in HSPCs from FA patients dramatically rescued their *in vitro* clonogenic ability (P<10^{-4}). These data suggest this p53/p21 response restricts the cell cycle in HSPCs, and that this mechanism could be central in BMF pathogenesis in FA patients.Although *Fancd2-/-* mice do not develop spontaneous BMF, they exhibit HSPCs defects. This defect was rescued in *Fancd2-/-p53-/-* mice (P<10^{-3}). Moreover, in *Fancg-/-* mice, shRNA-mediated p53 inhibition improved the Lin$^-$ cells engraftment in competitive experiments (P<10^{-4}). To study human cells, we lentivirally knocked-down *FANCD2* in CD34$^+$ cells from normal cord blood and xeno-transplanted the resulting FA-like cells into immunodeficient NSG mice. Strikingly, simultaneous knock-down of *p53* and *FANCD2* using tandem shRNA dramatically improved the engraftment capacities when compared to *FANCD2* shRNA (P=10^{-2}). Finally, gene expression analysis of fresh cells from FA patients and healthy donors showed that FA HSPCs strongly expressed the *p21/CDKN1A* gene (P<10^{-2}) and a signature of G0/G1 cell cycle arrest. **Summary and Conclusions.** Altogether, these results identify an exacerbated p53/p21-mediated G0/G1 cell cycle arrest in response to cellular stress as a central mechanism of BMF in FA patients. Noteworthy, a p53 response has also been involved in Diamond-Blackfan anemia and Dyskeratosis Congenita, related to ribosomal and telomeres abnormalities, respectively. Our findings in FA point to p53 activation as a unifying downstream signaling mechanism for inherited BMF syndromes.

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WHOLE EXOME SEQUENCING DEFINES CLONAL ARCHITECTURE AND GENOMIC EVOLUTION IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is an incurable malignancy of post-germinal centre B-cells whose pathogenesis is only partially understood. Chromosomal abnormalities, such as hyperdiploidy and recurrent immunoglobulin gene locus rearrangements are frequent, but are thought to be insufficient for malignant transformation because they are also observed in the pre-malignant syndrome called monoclonal gammopathy of uncertain significance (MGUS), while malignant progression is often associated with additional events, like MYC activation. This implies that the genetic landscape of MM changes over time as result of additional events that define its emergence and progression.To investigate genomic event underlying MM pathogenesis and evolution, we used targeted capture and massively parallel sequencing of exomes of CD138 purified plasma cells from 68 patients with MM. For 17 patients, serial samples (range 5-18 months apart) were available. Exome reads were used to call substitutions and insertions/deletions. We used single nucleotide polymorphisms (SNPs) to infer chromosomal copy number and allelic ratio of the tumor. Lastly, mutation burden was used to estimate the clonal architecture of each sample and its evolution over time (where available). All data were produced by in-house algorithms developed at the Wellcome Trust Sanger Institute.Our analysis confirmed mutation recurrence in some of the genes previously identified (Chapman et al, Nature 2011): KRAS 25%, BRAF 13%, NRAS 13%, FAM46C 9%, TP53 9%, CCND1 1%. Interestingly, 3/9 BRAF mutations co-occurred with activating KRAS mutations in the same patient, raising important therapeutic implications. We also described several genes previously unreported in MM. So far, 1803 variants have been validated in the cohort (range 0-140 per patient). 292 genes are recurrent, suggesting a role in MM pathogenesis, and among these are genes involved in the NF-kB pathway, histone-modifying enzymes, cyclins and cyclin-dependent kinases. We also report mutations in deubiquitinating enzymes, protein tyrosine phosphatases, cytoplasmic proteins involved in Golgi, endoplasmic reticulum and vesicle protein trafficking, and known tumor suppressors such as PTEN and APC.Analysis of the clonal structure showed at least two subclones in 66/68 (97%) patients at diagnosis, suggesting that myeloma is a heterogeneous disease at presentation. The burden of at least some of the mutations changed over time in 12/15 patients (80%) with serial samples, highlighting ongoing clonal evolution. Interestingly, 2/2 NRAS mutations only appeared in the late sample, and 3/6 KRAS mutations increased their burden over time. Furthermore, copy number analysis showed that 9/15 (60%) patients also acquired additional chromosomal gain/deletions, with loss of 17p in 4/9 (44%). This suggests a role for RAS activation and TP53 loss in disease progression.In conclusion, in our cohort of MM samples we show: 1) a comprehensive list of previously unreported variants, many of which are recurrent; 2) evidence of tumor heterogeneity at the time of diagnosis; 3) discernable genetic changes and shifts in the clonal structure of disease at the time of progression. Our study provides new insights into the molecular pathogenesis of MM, and will help identify molecular alterations associated with progression of disease and development of drug resistance.

0572

THE NANOBODY® ALX-0681 IS EFFICACIOUS AND SAFE IN THE PREVENTION AND TREATMENT OF THE ACUTE EARLY EPISODES OF ACQUIRED TTP IN BABOONS

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Background. Acquired thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening disorder. The disease is characterized by the deficient processing of ultra-large von Willebrand Factor (ULvWF) due to neutralization of ADAMTS13-activity. This leads to spontaneous platelet aggregation in the microvasculature responsible for the severe clinical symptoms of TTP. ALX-0681 is a therapeutic Nanobody that targets the A1-domain of vWF and inhibits the interaction between ULvWF and the platelet receptor GPIb-IX-V. Given the crucial role of ULvWF in the pathophysiology of TTP, inhibition of microthrombi formation by ALX-0681 offers an attractive new therapeutic concept for the treatment of acquired TTP. **Aims.** This study evaluated the efficacy and safety of ALX-0681 in a preclinical baboon model of acquired TTP, as described earlier (Feys *et al.*, Blood 2010, 116(12):2005-2010). **Methods.** ALX-0681 was evaluated for its potential to prevent or treat acute episodes of TTP induced by administration of an ADAMTS13-inhibiting monoclonal antibody (3H9). Onset of disease was demonstrated as rapid decreases of platelet counts and haptoglobin, and increases in LDH and schistocytes as major hallmarks of TTP. In the preventive setting, animals were treated with daily ALX-0681 injections starting on the day of the first 3H9 administration. In the therapeutic setting, animals received ALX-0681 only after confirmed onset of disease starting on day 5, after which daily dosing with the drug was ensued for the remainder of the study until day 11. **Results.** ALX-0681 completely prevented the rapid onset of thrombocytopenia after 3H9 injection when given before the onset of disease. Also intravascular hemolysis (indicated as decreasing haptoglobin levels), red blood cell fragmentation (increasing schistocyte count) and increasing LDH levels (tissue damage) were fully prevented. ALX-0681 also effectively treated the major hallmarks of acquired TTP. ALX-0681 completely reversed platelet counts to baseline levels. Schistocytic hemolytic anemia was halted as shown by the (partial) recovery of haptoglobin concentrations and stabilizing schistocyte counts, which showed a tendency towards normalization to baseline values. Another marker showing ALX-0681-induced normalization included LDH. Therapeutic efficacy of ALX-0681 fully correlated with complete neutralization of vWF activity as measured by ristocetin-induced cofactor activity. Importantly, brain CT scans during in-life phase and post mortem analysis of the organs did not reveal any sign of bleeding, irrespective of the observed reversible decreases of factor VIII clotting activity and vWF antigen concentrations.*Summary/* **Conclusions.** This study in the baboon model of acquired TTP convincingly demonstrated that ALX-0681 can prevent and treat the major hallmarks of acquired TTP by targeting the vWF-platelet interaction. Treatment was found to be safe in terms of bleeding risk even in the context of low platelet counts and full neutralization of vWF activity. Therefore, these preclinical data further strengthen the promising novel concept of inhibiting ULvWF-mediated platelet aggregation as adjunctive treatment for TTP in the clinic. ALX-0681 currently is undergoing Ph II clinical trials in TTP.

Novel technologies in acute lymphoblastic leukemia

0573

WHOLE GENOME SEQUENCE ANALYSIS OF 22 MLL-R INFANT ALLS REVEALS REMARKABLY FEW MUTATIONS-A REPORT FROM THE ST JUDE CHILDREN'S RESEARCH HOSPITAL AND WASHINGTON UNIVERSITY PEDIATRIC CANCER GENOME PROJECT

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Infant acute lymphoblastic leukemia (ALL) is characterized by rearrangements of the *MLL* gene (*MLL*-R) and a poor prognosis. We performed whole genome sequencing (WGS) on diagnostic leukemia and matched germ line samples from 22 infants with *MLL*-R ALL.Analysis of the structure of the *MLL*-R revealed that over half had complex rearrangements that involved either three or more chromosomes, carried cryptic rearrangements, or contained at the breakpoints deletions, amplifications, insertions, or inversion of sequences. In two of the complex cases, chromosomal rearrangements generated in addition to the *MLL-partner* gene fusion, novel in-frame fusions including *KRAS-MLL* and *AFF1-RAD51B-MLL*.An analysis of the non-silent variants (SNVs) revealed infant ALL to have the lowest frequency of somatic mutations of any cancer sequenced to date. After removal of structural variations (SVs) and copy number alterations associated with the *MLL*-R, and mutations present in minor subclones, the major clone in infant ALL contained a mean of only 3.5 SVs and 1.3 SNVs affecting the coding region of annotated genes or regulatory RNAs. Despite the paucity of mutations, several pathways were recurrently targeted including the PI3K/RAS pathway in 45% (*KRAS,* n=4, *NRAS,* n=2), and non-recurrent mutations in *NF1*, *PTPN11*, *PIK3R1*, and *ARHGAP32*,cell differentiation in 23% of cases as a result of mono-allelic deletion or gains of *PAX5*, 14% with deletions of *CDKN*, and 2 cases with focal deletions of the non-coding RNA genes *DLEU1/2*.WGS of two infant ALL relapse samples and comparison with their matched diagnostic samples revealed a marked increase in the number of mutations at relapse. Moreover, an analysis of the allelic ratios of mutated genes revealed clonal heterogeneity at diagnosis with relapse appearing to arise from a minor diagnostic clone.Because of the exceedingly low frequency of mutations detected in infant ALL, we determined the frequency of non-silent SNVs in *MLL*-R leukemia in older children (7-19 yrs). Exome sequencing on 20 *MLL*-R leukemias (9 ALLs, 10 AMLs, 1 AUL), revealed that non-infant pediatric *MLL* leukemias harbor a significantly higher number of non-silent somatic SNVs than infant ALL (mean 6.3/case in older patients versus 1.3/case in infants, $p<0.001$). In addition, genes involved in histone modification were mutated in 35% of non-infant leukemia. Although the increased frequency of mutations may be a reflection of the older age, the low number of cooperating mutations in infants raises the possibility that the target cell of transformation differs between infants and older children, with the cells present during early development requiring fewer cooperating mutations to induce leukemia.In summary, our data demonstrated an exceedingly small number of mutations in infant leukemia. The number of detected somatic mutations may represent the lower limit required to transform a normal human cell into a cancer. The lack of mutations in chromatin modifier genes in infant ALL raises the possibility that the target cell of transformation may differ between infants and older children, with the target cell in infants having a chromatin state that is more permissible to transformation by the *MLL* gene rearrangement.

0574

WHOLE TRANSCRIPTOME SEQUENCING IDENTIFIES NEW GENE REARRANGEMENTS IN T-ALL

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Background. T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of T-cell precursors that occurs predominantly in children and adolescents. Chromosomal rearrangements resulting in the overexpression of transcription factors such as TLX1, TLX3, TAL1, LMO1, LMO2 or HOXA are frequently observed in T-ALL, but gene fusions have been less commonly described. **Aims.** To discover new oncogenic lesions in T-ALL, we used whole transcriptome sequencing (RNA-seq) in T-ALL samples and T-ALL cell lines. **Methods.** Total RNA from twenty-one T-ALL cases (7 children and 14 adults), 16 T-ALL cell lines and normal thymus cells was analyzed by whole transcriptome sequencing. Next generation sequencing libraries were constructed from the mRNA fraction, followed by paired-end sequencing on a HiSeq2000 (Illumina). Sequence reads were aligned to the reference genome, and were processed to identify gene fusion transcripts and gene expression levels. Novel candidate fusion transcripts were confirmed by RT-PCR and Sanger sequencing. **Results.** Whole transcriptome sequencing identified an average of 38 fusion transcripts in each primary T-ALL case (range: 4-73) and 56 in each T-ALL cell line (range: 15-98).As expected, we identified a variety of fusion transcripts already reported in T-ALL, including NUP214-ABL1 (n=3), TRBC2-NOTCH1 (n=1), MLL-FOXO4 (n=1), PICALM-MLL10 (n=1) and SIL-TAL (n=4), or fusions known in other malignancies such as the NUP98-SPSI fusion (n=1). Interestingly, we also detected fusion transcripts between the T-cell receptor gene (TCR) and the TLX1 gene in 1 T-ALL cell-line, which also showed TLX1 overexpression in our RNA-seq gene expression analysis. Moreover, we detected and confirmed the presence of novel fusion transcripts. Several novel in-frame fusion transcripts involving transcriptional regulators were identified, including the CLINT1-MEF2C, HNRP-ZNF219 (n=2), FUS-SET and DDX3X-AF10 fusions, and we detected 2 novel fusion transcripts involving the kinase domains of FER and JAK2. Furthermore, new translocations involving the TCR genes were discovered. These translocations resulted in the generation of fusion transcripts between the TCR genes and genes on the partner chromosome, and were accompanied by the clear overexpression of one or more genes on the partner chromosome. In this way, we identified chromosomal rearrangements between the IL7R gene and TRBC2, between RIC3 and TRBC2 (also causing LMO1 overexpression), and between SFT3 and TCRD (also causing NKX2-1 overexpression).Out-of-frame fusion transcripts were detected involving TP53, IKFZ1 and CDKN2A, which could be a mechanism to inactivate these tumor suppressor genes. **Conclusions.** Our study demonstrates the power of whole transcriptome analysis for the detection of fusion transcripts and overexpressed genes in T-ALL. Using a combined analysis of fusion transcript detection and gene expression levels, we were able to detect translocations involving the TCR genes, in-frame-fusion transcripts and out-of frame fusion transcripts that may cause inactivation of tumor suppressor genes.

0575

EXOME SEQUENCING OF LATE RECURRENCE T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN CONFIRMS SECOND LEUKEMIA AND IDENTIFIES PREDISPOSITION CANDIDATE GENES

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Background. Second hematologic malignancies in non-syndromic children without family history for cancer may be mistaken for relapses or therapy-related malignancies. Recently, we identified eight T-cell acute lymphoblastic leukemia (T-ALL) patients with two consecutive leukemias fully discordant for TCR-rearrangements and DNA copy number aberrations, strongly suggesting a predisposition to leukemia (J.Clin.Oncol.2011). **Aims and method**Here, we performed exome sequencing on leukemic and complete remission samples from four of these patients in order to assess clonal relationship at the sequence level, and to search for predisposing mutations. **Results.** We found an average of 25,000 variants/exome. All variants that were present in public (dbSNPv32) and in-house (672 exomes) databases were excluded for further analysis, as well as all synonymous variants or intronic variants, and those called in <20% of the reads. These filter steps resulted in an average of 430 private variants per exome.All candidate somatic variants shared between two consecutive leukemic samples were re-sequenced by Sanger sequencing and were shown to be either present in all three samples, and thus originally missed in the remission sample, or falsely detected in one or more leukemic samples. Therefore, we conclude that no somatic variants were shared between the first and second leukemic presentations, which confirms that these patients suffered from clonally unrelated second T-ALLs. From all somatic variants present in only one of the leukemic samples, we focused on variants in exons or splice junction sites. We identified and validated between one and six somatic variants per leukemic sample, the majority of which affected known T-ALL genes, such as *PTEN, FBXW7* and *PHF6*. In order to identify candidate predisposing constitutional variants, we focused on recurrently affected genes and known T-ALL associated genes. We identified highly conserved missense variants in *TYK2, RANBP17,* and *TIAL1*. *TYK2* was also affected by somatic mutations and belongs to the family of Janus kinases, which play a role in the pathogenesis of several hematologic malignancies. The germline *TYK2* variant G761V is located in a highly conserved region of the pseudokinase-like domain, which is frequently affected in the homologous *JAK2* kinase in precursor B-cell leukemias. **Conclusions.** We confirmed second leukemia in four patients with late T-ALL recurrences, and identified the tyrosine kinase *TYK2* as a candidate predisposing gene.

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UNRAVELING A NOTCH1-LNCRNA-MIRNA REGULATORY NETWORK IN ACUTE T-CELL LYMPHOBLASTIC LEUKEMIA AND NORMAL T-CELL DEVELOPMENT

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Background. NOTCH1 signaling is of central importance in normal development and differentiation. NOTCH1 acts as a central player in oncogenesis of several cancer entities, in particular T-cell acute lymphoblastic leukemia (T-ALL). T-ALL is a hematological malignancy, characterized by uncontrolled proliferation and arrested differentiation of precursor T-cells. Activating *NOTCH1* mutations are present in more than 50% of all T-ALL patients. Although the protein coding regulatory network governed by NOTCH1 is extensively characterized, its function in non-coding networks remains largely unexplored. **Aims.** In this study, we aim to identify long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) whose activities are controlled by NOTCH1 in normal and malignant T-cell development and functionally validate relevant candidate lncRNAs and miRNAs both *in vitro* and *in vivo*. **Methods.** Using a γ-secretase inhibitor (compound E) interfering with NOTCH1 signaling in four T-ALL cell lines (JURKAT, HPB-ALL, DND-41 and T-ALL1), transcriptional response of all protein coding genes and 8000 lncRNAs was assessed using gene expression microarrays (Agilent). In parallel, lncRNA expression profiles were also established for two sorted normal T-cell progenitor populations derived from either CD34+ cord blood or thymus cells cultured either with or without NOTCH1 stimulation using OP9-DL1 or OP9-GFP feeder layers. Finally, a top-50 ranked list of highest expressed lncRNAs in eight different T-ALL cell lines was generated upon RT-qPCR profiling of 1250 lncRNAs (Biogazelle). In addition to lncRNAs, the miRNAome (756 miRNAs) for all experimental conditions as well as nine distinct sorted subsets of normal developing T-cell populations and 20 T-ALL cell lines were profiled using high-throughput stem-loop qPCR (Applied Biosystems). Expression of known NOTCH1 protein coding target genes were in keeping with the expected response validating our procedure. **Results.** Using a unique integrated experimental approach, we were able for the first time to identify a subset of lncRNAs and miRNAs acting downstream of the NOTCH1 signaling cascade with a presumed function in both normal and malignant T-cell development. Differentially expressed lncRNAs and miRNAs between untreated (DMSO) and treated (compound E) conditions for each GSI-sensitive cell line (DND-41, HPB-ALL and T-ALL1) were scored in a time course experiment against the GSI-resistant cell line (Jurkat). In total, we identified 5 NOTCH1 neg-

atively regulated lncRNAs and 24 NOTCH1 positively regulated lncRNAs shared amongst the three GSI-sensitive cell lines. Publicly available NOTCH1 ChIP-seq data were used to search for direct NOTCH1 binding to the promoters of regulated lncRNAs (Aster et al., PNAS, 2011). A subset of these candidate lncRNAs will be further evaluated for their physiological role *in vitro* by means of overexpression or siRNA-mediated knockdown. **Conclusions.** We applied a unique experimental approach that enabled the first landscaping of a comprehensive integrated network of lncRNAs and miRNAs acting downstream of the NOTCH1 signaling pathway in T-ALL and normal T-cells. Given the central role of NOTCH1 in T-ALL oncogenesis, these data pave the way towards development of novel therapeutic strategies impacting on hyperactive NOTCH1 signaling.E-mail address: franki.speleman@ugent.be

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PROTEOMIC EXPLORATION OF CELL SURFACE LANDSCAPE REVEALS NEW LEUKEMIA ASSOCIATED FEATURES

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Background. Proteins present on the cell surface constitute a gateway through which the cell can communicate with the environment. In leukemia, such surface proteins play a role in a wide range of events including cell homing and protective interactions with the microenvironment, which might lead to chemoresistance. Thus, describing the leukemia cell surface landscape will provide a resource to study the biology of this disease and to identify new leukemia-associated proteins as potential diagnostic markers and treatment targets. **Aims.** Here we combined a leukemia xenograft model with mass spectrometry based techniques in order to identify new leukemia associated cell surface proteins. **Methods**. Amplification of primary human material by xenotransplantation allowed generating highly viable samples of sufficient size to apply the mass spectrometry-based Cell Surface Capturing (CSC) technology that enables specific detection of glycoproteins exposed at the cell surface. We have analyzed 19 samples of precursor B-cell acute lymphoblastic leukemia (ALL), including cases with a very high risk of relapse and cases with very good prognosis, based on the levels of minimal residual disease (MRD) during treatment. This cohort composition enabled us to detect surface proteins that are expressed in most of ALL cases, but also to look for markers associated with *in vivo* resistance to treatment. **Results.** As a proof of concept, the immunophenotype established at diagnosis by flow cytometry was readily recapitulated in our proteomic dataset. In total, almost 1000 membrane-associated proteins were identified on the surface of ALL cells. Among these, we identified 234 proteins being consistently present on more than 60% of the samples. Taking advantage of recent comprehensive gene expression data from sorted cell populations of the normal human hematopoietic tree (DMAP, Novershtern et al, 2011, Cell, 144, 296-309), we filtered our candidate list for proteins which are not expected to be detectable at early stages of B-cell ontogeny. For 38 of these proteins, mRNA levels were very low also in sorted populations from other normal B lineage cells. Among those, we found surface proteins that have been detected by other approaches as useful markers for the distinction of leukemic blasts from their normal counterparts, such as CD99, IL3RA, CD300A and CD58. We are currently evaluating 15 new promising leukemia associated proteins by flow cytometry in ALL samples at diagnosis and during induction chemotherapy.Additionally we filtered our proteomic dataset for proteins that are detected preferentially in cases with very high-risk leukemia. Interestingly, this approach generated a limited list of 37 candidates, including two members of the vanin family (VNN), with proposed functions in hematopoietic cell trafficking. Validation studies showed that high expression of VNN2 identifies a small subgroup with positive MRD in a cohort of 600 patients, indicating that this protein may recognize a subset of ALL cases with more unfavorable biology. **Conclusions.** Taken together, our data provide an unprecedented view at the cell surface landscape of leukemia cells and identify subsets of cell surface proteins that could be amenable for diagnostic and prognostic use or therapeutic intervention in this deadly disease.

Chronic lymphocytic leukemia - Translation research

0578

DEEP SEQUENCING IDENTIFIES TP53 MUTATIONS BEFORE THEIR CLONAL SELECTION BY THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Defects in the *TP53* gene are associated with a particularly adverse prognosis in chronic lymphocytic leukemia (CLL). The p53 mutation and/or 17p deletion effect a poor chemotherapy response and shorter clinical responses to chemoimmunotherapy or alemtuzumab. The frequency of p53 inactivation is substantially higher in pretreated cohorts compared to those investigated before first-line therapy. This raises questions on the role of treatment in *TP53* mutagenesis and specifically whether therapy only selects mutations or induces them *de novo*. **Aims.** The aims were to analyze i) frequency of new clonal *TP53* mutations in untreated and treated CLL patients ii) in cases with new clonal *TP53* mutation emerging after therapy, to analyze its presence before therapy using the ultra-sensitive deep sequencing. **Methods.** Clonal p53 mutations were identified by the yeast functional analysis (FASAY) and/or by direct Sanger sequencing. Presence of 17p- was assessed by I-FISH using probe from Abott-Vysis. The pyrosequencing on the GS Junior 454 platform (Roche) was performed to identify low-proportion (yet unselected) *TP53* mutations. For the positions of the studied mutations, we achieved a median coverage of 6 657 reads (range 5 212-16 427). Mutations were reliably detected with the sensitivity reaching 0.2%. **Results.** We have repeatedly analyzed p53 status in 248 CLL patients which had an intact *TP53* gene (no mutation and no 17p-) at first investigation; the median follow up was 33 months during which 60% of patients received therapy. The repeated investigations identified 31 patients with new p53 mutation (12.5%). All these mutations have emerged exclusively in patients that received treatment prior to detection of the p53-mutated clone; approximately each fifth treated patient acquired a new p53 mutation. Remarkably, missense mutations located in the p53 DNA-binding motifs (DBMs) - well defined and structurally important parts of the DNA-binding domain - were preferentially selected by therapy. Deep sequencing of pre-therapy samples from 9 patients that subsequently developed p53 mutation has revealed that all mutations were present as minor clones undetectable by standard techniques (range 0.24% -13.03% of mutated reads) ($P < 0.001$); all mutations were identified from both the forward and reverse primers. After the therapy, all analyzed mutations were clearly detectable by deep-sequencing in a much larger proportion of cells. Our data shows that therapy selects "ready-to-use" mutations; by other words, there is probably none or negligible *de novo* induction of new *TP53* mutations by therapy in CLL. **Summary and Conclusions.** We demonstrate that selection of very minor clones of CLL cells harboring *TP53* mutations is likely connected to the relapse of the disease and is a key event in evolution of certain refractory CLL cases. The detection of minor clones of CLL cells with p53 mutation prior to therapy could be of potential relevance for therapy selection and subsequent management of the disease relapse. This work was supported by the projects CZ.1.05/1.1.00/02.0068, CZ.1.07/2.3.00/20.0045, CZ.1.07/2.4.00/17.0042, and MSM0021622430.

0579

INCREASED DISEASE SPREADING THROUGH CD49D/ALPHA4 INTEGRIN AND APOPTOSIS BLOCK MEDIATED BY CD69 REPRESENT TWO KEY PROGNOSTIC PARAMETERS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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CD49d-over-expressing CLL cells demonstrate enhanced migration properties spreading in peripheral lymphoid organs, thus characterizing patients with a more advanced disease (Gattei, 2008). On the other hand, CD69 overexpression reflects ongoing *in vivo* stimulation and increased intracellular sig-

nalling, thereby explaining a more aggressive disease (Del Poeta, 2012). Moreover, up-regulation of CD69 in CLL B-cells was associated with reduced spontaneous apoptosis and drug-induced apoptosis (Ding, 2009). On this line, the actual therapeutic availability both of a humanized anti-CD49d monoclonal antibody (natalizumab) and of small pro-apoptotic molecules prompted us to evaluate CD49d and CD69 expressions in a large number of CLL cases accrued at our Institution. The primary endpoints of our study were: 1) to test the associations between CD69 or CD49d and clinical/biological features; 1) to determine time to first treatment (TTFT) and overall survival (OS) upon CD69 and CD49d; 2) to assess the additive prognostic value of CD69 and CD49d and finally 3) to confirm CD69 and CD49d as independent prognostic factors. We investigated 528 pts, median age 65 years, 294 males and 234 females. The informed consent was obtained in accordance with the declaration of Helsinki. With regard to modified Rai stages, 171 had a low stage, 338 an intermediate stage and 19 a high stage. CD49d and CD69 antigens were determined by multicolor flow cytometry (FACSCalibur, BDIS), fixing the cut-off of positivity at 30%. CD69+ and CD49d+ pts were 129/484 (27%) and 186/425 (44%), respectively. CD69 and CD49d were significantly correlated with Rai stages (P<0.0001), beta-2 microglobulin (P=0.00003 and P=0.00002) and soluble CD23 (P<0.0001 and P=0.0005). Lymphocyte doubling time > or <12 months was correlated only with CD69 (P<0.0001), while 22/26 cases (85%) with a bulky disease (>10cm) were CD49d+ (P=0.00002). Noteworthy, CLL patients with splenomegaly and/or thoracic/abdominal lymphadenopathies >3cm were more frequently CD49d+ (96/150; P<0.0001). With regard to biological findings, CD69 >30% was significantly associated with a low bax/bcl-2 ratio (P=0.0006), confirming that upregulation of CD69 means to block spontaneous apoptosis. Interestingly, CD49d >30% was significantly correlated with trisomy 12 (44/65) and del17p (10/15) [P=0.00005]. Both CD69+ and CD49d+ cases were associated with IgVH unmutated status (P=0.00004 and P=0.0007). With regard to clinical outcome, shorter TTFT (P<0.0001, Figure) and OS (P<0.0001 and P=0.001) were observed both in CD69+ and CD49d+ pts. Noteworthy, CD69 and CD49d showed additive prognostic properties, since CD49d >30% plus CD69 >30% identified a CLL subset at worst prognosis with regard to TTFT (Chi-square: 56.94; P<0.00001, Figure) and OS (Chi-square: 14.32; P=0.002). The two discordant subsets (CD69+CD49d- and CD69-CD49d+) showed an intermediate outcome. In multivariate analysis of TTFT, CD69 (P=0.0006) and CD49d (P=0.03) together with cytogenetics, ZAP-70 and IgVH status were confirmed to be independent prognostic factors. In conclusion, CD69 and CD49d over-expressions may identify two different subsets of progressive CLL patients, the first characterized by a high proliferation rate and block of apoptosis, the second mainly by a large volume of disease. Their combined flow cytometric determinations at diagnosis are useful to better characterize these progressive CLL patients, directing a more targeted therapy.

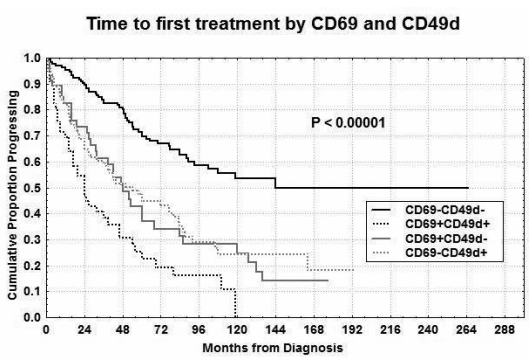

Figure 1.

0580

DISTINCT PROFILES OF MIRNAS MODULATING IMMUNE SIGNALLING PATHWAYS IN DIFFERENT SUBSETS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Stimulation through the B-cell receptor (BcR) is critically implicated in CLL ontogeny. However, mounting evidence suggests that other modes of stimulation, acting in concert with BcR-mediated signaling, can also be relevant. We have recently reported that different CLL subgroups defined by specific molecular characteristics of the clonotypic BcRs exhibit distinct expression profiles of the Toll like receptor (TLR) signaling pathway molecules as well as differential functional outcomes after TLR stimulation. Here, we investigated whether miRNAs could be implicated in the control of TLR and BcR signaling in CLL. Using customized cDNA arrays, we profiled negatively selected $CD19^+$ B cells from 79 CLL cases for the expression of 34 miRNAs, which were selected through bioinformatics analysis on the basis of the following criteria: (i) deregulated in CLL compared to normal B-cells; (ii) differentially expressed in CLL subgroups with distinct clinical and biological features; and, (iii) if meeting (i)+(ii), having potential targets in the BCR and TLR pathways. miR-150 displayed the higher expression level among all miRNAs studied; high expression was also recorded for 7 other miRNAs, including miR-16 and miR-29a; intermediate expression for 5 miRNAs, including miR-101; low expression for 21 miRNAs, including several members of the 17-92 cluster, miR15a and miR15b. For certain miRNAs, great variability was observed among different CLL cases, prompting us to compare miRNA profiles in subgroups of cases defined by (i) IGHV gene mutational status and (ii) BcR stereotypy. The analysis revealed significant upregulation of miR-150 and miR-223 and downregulation of miR-15a in mutated vs. unmutated CLL cases, with miR-15a showing the highest fold difference overall. Through bioinformatics search, we identified JNK as the highest scoring potential target of miR15a in the BcR and/or TLR pathways and further investigated its expression by RQ-PCR and Western Blotting. We noted a significant anti-correlation ($p<0.01$) between miR-15a levels and JNK mRNA expression levels. Furthermore, preliminary data show that mutated CLL cases express higher JNK protein levels compared to unmutated CLL cases. We next compared two different subsets with stereotyped BcRs, namely subset #1 (IGHV1/5/7-IGKV1(D)-39, unmutated) vs subset #4 (IGHV4-34/IGKV2-30, mutated). These subsets are prototypes of unmutated/adverse prognosis and mutated/good prognosis CLL, respectively, and have also been shown to exhibit distinct signalling signatures. The most pronounced difference concerned miR-101, which was found up-regulated in subset #4. miR-101 is considered as an «epi-miRNA» since it targets EZH2, which mediates transcriptional repression through histone methyltransferase activity. Interestingly, EZH2 mRNA and protein levels were found downregulated in subset #4 vs #1, indicating that miR101 is functionally competent in this setting. With this in mind, we next investigated whether it may also modulate immune signalling in these subsets. We focused on c-FOS, the highest scoring predicted target of miR101 in the BcR and/or TLR pathways, and obtained evidence for significant anti-correlation between miR101 levels and c-FOS mRNA levels, whereas no correlation existed at the protein level. In conclusion, certain miRNAs differentially expressed among CLL subgroups with distinct BcRs may modulate immune signaling, eventually affecting the biological behavior of the CLL clones.

0581

CMV-SPECIFIC T CELL FUNCTION IS NOT IMPAIRED IN CLL

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Introduction. The interaction between CLL cells and T cells is a dynamic and reciprocal process in which the exact impact on T cell function is ill-defined. Although T cell numbers are expanded, several lines of evidence showed impaired T-cell function, reflected both in disturbed immunological synapse formation and defective cytotoxic capability. Paradoxically, clinically relevant reactivation of latent viruses such as CMV is uncommon, especially in untreated patients. Whether the general disturbed defects in T cell homeostasis and function also applies to the subset of virus-specific T cells is currently unknown. **Aims.** To study consequences of CLL tumor burden on CMV-specific T cells **Methods.** T-lymphocyte subsets; i.e. naive, memory and effector-(memory) of $CD4^+$ and $CD8^+$ cells of 32 MBL, 26 CLL and 7 age-matched healthy donors (HD) were assessed by FACS analysis. Whole blood CMV-PCR was performed on 22 untreated CLL patients (median lymphocyte count XX (range XX-XX). $CD8^+$ CMV specific cytokine production was determined by FACS analysis on intracellular IFNγ, TNFα and IL-2 production of CMV-specific T-cells, 6 hours after stimulation with EBV-positive lymphoblastoid cell line derived from normal B lymphocytes loaded with CMV-pp65 NLV peptides. Additionally, $CD8^+$ CMV specific cytotoxicity against loaded EBV-positive lymphoblastoid cells was determined by FACS. Immunological synapse formation is assessed by Image Stream analysis. **Results.** $CD4^+CD28^-CD45RA^+$/- (effector) and $CD8^+CD27^-CD45RA^+$/- (effector-memory) T cells were found to be significantly expanded only in CLL patients with a B-lymphocyte count over $10x10^9$/L. This expansion was mainly seen in patients with positive CMV+ serology. All of these patients appeared to have undetectable CMV viral loads. To study whether the intact T-cell mediated CMV responses are attributed to the reported increased quantity of functionally disturbed effector-memory type $CD8^+$ cells or that function is maintained in this subset of cells we performed cell-to-cell comparative analyses. CLL derived CMV-specific T cells were able to produce equal amounts of IFNγ, TNFα and IL2 compared to HD-derived CMV-specific T cells, provided that they were stimulated with adequate antigen-presenting cells (APC) like CMV-peptide EBV-positive lymphoblastoid cell line. Moreover, CMV-specific cytotoxicity of CLL CMV-specific T cells and HD CMV-specific T cells started to occur at E:T ratios of 1:16 in equal amounts and reached a maximum at an E:T ratio of 2:1 (specific lysis 71,8% (95%CI 61,5-82,0) and 74,0% (95%CI 66,4-81,6) respectively). Currently, both qualitative and quantitative comparison of immunological synapse formation is performed by Image Stream analysis and will be presented. **Conclusions.** In recent years, putative systemic T cell dysfunction inCLLpatients has received increasing attention. This study shows that CLL specific alterations in T-cell subsets do not follow the defined cut-off of MBL and CLL. Despite evidence for disturbed immunological synapse-formation and cytotoxicity in general in T cells, our study is the first to show that T cell mediated immunity against latent viruses remains intact in this disease.

0582

CYTOGENETIC ABERRATIONS IN THE CD38 POSITIVE FRACTION OF CD38 NEGATIVE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS: A MARKER OF AGGRESSIVENESS?

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Background. Chronic lymphocytic leukemia (CLL) is a disease with a high variability in clinical presentation and outcome. Even though many patients live for long periods with the disease, some cases may show a progression to a more aggressive leukemia which may be characterized by the acquisition of new genetic abnormalities either by clonal evolution or by an expansion of a clone with high risk aberrations. Prognostic parameters that correlate with worse clinical outcome include stage, the expression of CD38 and/or ZAP70, the unmutated configuration of the variable region of the immunoglobulin heavy chain gene (IGHV), the presence of specific chromosome aberrations and/or molecular mutations affecting TP53, NOTCH1, SF3B1 and BIRC3. However in patients with favourable prognostic features the biologic and molecular events leading to disease progression and the occurrence of new molecular cytogenetic lesions are largely unknown. **Aims**. We studied the biologic and clinical significance of minor cytogenetically abnormal clones in the CD38 positive fraction of untreated CLL patients with good prognostic parameters (CD38 negativity and normal karyotype or del(13)(q14) as single aberration). **Methods**. Twenty eight consecutive CD38 negative (Cd38 positive cells < 5%) CLL patients with normal karyotype or del(13)(q14) were evaluated in this study. CD38 positive and CD38 negative CLL cells were isolated by sequential immunomagnetic sorting (Dynabeads) following depletion of CD3, CD16, CD14 positive cells (purity > 99%). CD38 positive and negative cells were then analyzed by (i) FISH analysis using commercially available probes for the regions most frequently involved in CLL patients (13q14, 12q13, 11q22/ATM, 17p13/TP53, 14q32) and by (ii) micro-RNA expression. Data were then correlated with clinical parameters. **Results**. In 16/28 CD38 negative CLL patients, FISH analysis demonstrated the presence of minor (15-34% of the cells) cytogenetically abnormal clones within the CD38 positive fraction: 11q deletion in 7 cases, 17p deletion in 6, trisomy 12 in 5, 14q32 rearrangements in 1 case. According to FISH results patients were therefore classified as CLL with and without subclones in the CD38 positive fraction. By micro-RNA analysis we found that patients with subclones had a distinctive profile, when compared to patients without subclones, characterized by a downregulation of microRNA-125a-5p (a tumor suppressor in various malignancies) both in the CD38 negative and positive populations. With a median follow-up of 36 months, patients with subclones showed a higher need of treatment (9/16 cases vs 1/11 in patients with and without subclones respectively, p=0.0159). Among CLL patients with subclones, 2 cases showed the emergence of a major clone in the peripheral blood sample (45-67% of the cells) with the same genetic lesions previously observed in the CD38 positive subpopulations.ConclusionsOur data showed that genetic instability within the CD38 positive fraction of CLL patients with favourable prognostic features (CD38 negativity and normal karyotype or del13q14) may favour the growth of small clones with poor prognosis cytogenetic aberrations which may be associated with microRNA-125a-5p disregulation, clonal expansion and, in some cases, disease progression.

First line trials in chronic myeloid leukemia

0583

SUPERIOR EFFICACY OF NILOTINIB COMPARED WITH IMATINIB IN NEWLY DIAGNOSED PATIENTS WITH PH+ CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENESTND 3-YEAR FOLLOW-UP

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Background. At 2 years of follow-up from the landmark ENESTnd study, nilotinib significantly reduced progression to accelerated phase/blast crisis (AP/BC) and demonstrated superior rates of major molecular response (MMR; ≤ 0.1% BCR-ABL^{IS}), molecular response with a 4-log (MR^4, ≤ 0.01%^{IS}) or 4.5-log (MR$^{4.5}$, ≤ 0.0032%^{IS}) reduction in BCR-ABL transcript levels vs imatinib. **Aims.** Here, we report results at a minimum follow-up of 3 years. **Methods.** A total of 846 patients with newly diagnosed Ph+ CML-CP were randomized to nilotinib 300 mg twice daily (BID) (n = 282), nilotinib 400 mg BID (n = 281), or imatinib 400 mg once daily (QD) (n = 283). MMR, MR^4, MR$^{4.5}$, time to progression to AP/BC on treatment and after discontinuation, overall survival (OS), and treatment-emergent mutation data are reported.

Table 1.

	Nilotinib 300 mg BID (n = 282)	Nilotinib 400 mg BID (n = 281)	Imatinib 400 mg QD (n = 283)
Rate of response by 3 years, %			
MMR	73 *P* < .0001	70 *P* < .0001	53
MR^4	50 *P* < .0001	44 *P* < .0001	26
MR$^{4.5}$	32 *P* < .0001	28 *P* = .0003	15
Estimated 3-year rate of freedom from progression to AP/BC, %			
On core treatment	99.3 *P* = .0059	98.7 *P* = .0185	95.2
Including events after discontinuation	98.7 *P* = .0496	98.1 *P* = .0076	93.5
Estimated 3-year rate of OS, %			
All deaths	95.1 *P* = .4413	97.0 *P* = .0639	94.0
Only CML-related deaths	98.1 *P* = .0356	98.5 *P* = .0159	95.2
Patients with BCR-ABL mutation, n			
Any mutation	11	11	21
Mutation category, n			
T315I	3	2	3
Less sensitive to nilotinib[a]	6	9	4
Other mutations[b]	2	0	14
Patients with new mutations by Sokal score, n			
Low (n = 103, 103, 104)	1	2	1
Intermediate (n = 101, 100, 101)	5	3	8
High (n = 78, 78, 78)	5	6	12
Response status, n			
Treatment failure[c]	4	6	16
Suboptimal response[c]	6	2	5
Confirmed loss of MMR[c,d]	0	2	0
Other[c,e]	1	1	0

[a] E255K/V, Y253H, and F359C/V.
[b] All mutations except E255K/V, Y253H, F359C/V, and T315I.
[c] Patients were only counted once under the worst-case response category.
[d] Loss of MMR was not considered a criterion for suboptimal response.
[e] The patient in the nilotinib 300 mg BID arm progressed after discontinuation of treatment, and the patient in the nilotinib 400 mg BID arm had an unconfirmed loss of MMR but regained MMR after that.
Confirmed cytogenetic response data were not collected after 2 years.

Results. Significantly higher rates of MMR, MR^4, and MR$^{4.5}$ were observed for both doses of nilotinib vs imatinib (Table 1). The difference in rates of MR^4 and MR$^{4.5}$ between nilotinib and imatinib increased over time. Rates of molecular response were superior for nilotinib, regardless of Sokal risk score. No new progressions occurred on treatment since the 2-year analysis. There was a significantly lower rate of progression to AP/BC on treatment for both nilotinib arms compared with the imatinib arm (n = 2, 3, and 12 in the nilotinib 300 BID, nilotinib 400 mg BID, and imatinib arms, respectively). When events that occurred both on treatment and after discontinuation were considered, the rate of progression to AP/BC remained significantly lower for both nilotinib arms compared with imatinib (n = 9, 6, and 19 in the nilotinib 300 BID, nilotinib 400 mg BID, and imatinib arms, respectively). At 3 years, OS was 95.1%, 97.0%, and 94.0% for nilotinib 300 mg BID, nilotinib 400 mg BID, and imatinib, respectively. There were significantly fewer CML-related deaths in the nilotinib 300 mg BID (n = 5) and nilotinib 400 mg BID arms (n = 4) vs imatinib (n = 14). Twice as many patients had emergent BCR-ABL mutations on imatinib (n = 21) vs nilotinib (n = 11 in each arm); 5 additional patients had emergent mutations since the 2-year analysis (1 on nilotinib 300 mg BID, 3 on nilotinib 400 mg BID , and 1 on imatinib). Mutations were more frequent in patients with high or intermediate Sokal risk, and most of these patients had suboptimal response or treatment failure. The incidence of T315I mutations was similar for the nilotinib (n = 3, nilotinib 300 mg BID; n = 2, nilotinib 400 mg BID) and imatinib (n = 3) arms. Both drugs were well tolerated. Since the 2-year follow-up, increases in hematologic/biochemical abnormalities and other adverse events have been minimal. **Conclusions.** With a minimum follow-up of 3 years, nilotinib continues to demonstrate a significantly lower rate of progression to AP/BC (both on treatment and including events after discontinuation), lower rates of mutations, and superior rates of MRs vs imatinib. These data continue to support nilotinib as a potential new standard of care in newly diagnosed patients with Ph+ CML-CP.

0584

EARLY BCR-ABL TRANSCRIPT LEVELS PREDICT FUTURE MOLECULAR RESPONSE AND LONG-TERM OUTCOMES IN NEWLY-DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE: ANALYSIS OF ENESTND 3-YEAR DATA

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Background. In the ENESTnd study, nilotinib significantly reduced progression to accelerated phase/blast crisis (AP/BC) both on treatment and including events occurring after discontinuation, and demonstrated superior rates of molecular response vs imatinib. Several independent groups have conducted analyses suggesting that achievement of BCR-ABL transcript levels according to the international scale (IS) of <10% and <1% (considered as equivalent to a complete cytogenetic response) at 3 months were associated with improved outcomes. **Aims.** Based on a landmark analysis of the BCR-ABL transcript levels at 3 months, we predicted the rate of future major molecular response (MMR; ≤0.1% BCR-ABL^{IS}), and molecular response with a 4.5-log (MR$^{4.5}$, ≤0.0032%^{IS}) reduction in BCR-ABL transcript levels at later time points, and also long-term outcomes including progression to AP/BC, and overall survival (OS). **Methods.** The nilotinib 300 mg BID (n = 282) and imatinib (n = 283) arms from ENESTnd were used for this analysis. In each treatment arm, patients were grouped based on BCR-ABL transcript levels of ≤1%, >1% to 10%, or >10% at 3 months (n = 258 and n = 264 patients with available PCR samples at 3 months in the nilotinib and imatinib arms, respectively). Rates of MMR and MR$^{4.5}$ as well as rates of progression to AP/BC and OS were evaluated among patients grouped according to their BCR-ABL transcript levels at 3 months. Patients who achieved the target response within 3 months (for response outcomes) or who had events or were censored within 3 months (for time-to-event outcomes) were excluded from the respective analysis. **Results.** Among evaluable patients at 3 months, 91% of patients in the nilotinib arm vs 67% in the ima-

tinib arm achieved BCR-ABL transcript levels of ≤10%; 56% vs 16% of patients achieved BCR-ABL transcript levels of ≤1%. With either treatment, the rates of MMR by 1 and 2 years and rate of $MR^{4.5}$ by 2 and 3 years were higher in patients who achieved BCR-ABL transcript levels of ≤1% than in patients who had either >1% to 10% or >10% BCR-ABL at 3 months (Table). The initial molecular response also correlated with progression to AP/BC and with OS. Of the 187 patients who achieved ≤1% BCR-ABL at 3 months, only 1 progressed on treatment (on imatinib); 2 of 222 patients who achieved >1% to 10% progressed (both on imatinib) and 9 of 111 patients who achieved >10% at 3 months progressed (1 on nilotinib and 8 on imatinib). Patients who achieved ≤1% BCR-ABL transcript levels at 3 months had a higher OS rate than patients who achieved >10% BCR-ABL transcript levels at 3 months (3-year OS 97% and 87%, respectively, in the nilotinib arm and 95% and 85%, respectively, in the imatinib arm). **Conclusions.** More patients in the nilotinib arm vs the imatinib arm achieved BCR-ABL transcript levels ≤1% (56% vs 16%) and ≤10% (91% vs 67%) at 3 months. Early molecular response at 3 months correlated with future MMR and $MR^{4.5}$ as well as an increased probability of freedom from progression and OS.

Table 1.

	Nilotinib 300 mg BID			Imatinib		
BCR-ABL at 3 months	≤ 1% N = 145	> 1% to 10% N = 89	> 10% N = 24	≤ 1% N = 43	> 1% to 10% N = 133	> 10% N = 88
MMR[a]	n = 120	n = 89	n = 24	n = 41	n = 133	n = 88
by 1 year (%)	76	40	4	71	31	2
by 2 years (%)	89	67	29	78	52	20
MR[4.5 a]	n = 144	n = 89	n = 24	n = 43	n = 133	n = 88
by 2 years (%)	40	12	4	33	8	0
by 3 years (%)	50	18	4	53	14	1
Without AP/BC on treatment[a]	n = 145	n = 89	n = 24	n = 42	n = 133	n = 87
at 1 year (%)	100	100	96	97	100	91
at 2 years (%)	100	100	96	97	98	90
at 3 years (%)	100	100	96	97	98	90
Without AP/BC on study[a,b,c]	n = 145	n = 89	n = 24	n = 43	n = 133	n = 88
at 1 year (%)	100	98	96	98	100	91
at 2 years (%)	99	98	91	98	99	86
at 3 years (%)	99	97	91	98	99	85
OS[a,b]	n = 145	n = 89	n = 24	n = 43	n = 133	n = 88
at 1 year (%)	100	100	96	100	100	98
at 2 years (%)	99	100	96	100	100	88
at 3 years (%)	97	99	87	95	100	85

[a] Evaluable patients (n) excludes patients with unevaluable/missing polymerase chain reaction assessments at 3 months, atypical transcripts at baseline, or those who achieved the target response within 3 months (for response outcomes) or who had events or were censored within 3 months (for time-to-event outcomes).
[b] Including events after discontinuation of treatment.
[c] Progression on study was defined as progression to AP/BC occurring during core treatment or after discontinuation of treatment during patient follow-up.

0585

FRONTLINE TREATMENT OF PHILADELPHIA POSITIVE CHRONIC MYELOID LEUKEMIA WITH SEQUENTIAL ADMINISTRATION OF NILOTINIB 400 MG TWICE DAILY AND IMATINIB 400 MG ONCE DAILY: A PHASE 2 MULTICENTRIC STUDY

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Background. The golden therapeutic standard for Philadelphia positive (Ph+) chronic myeloid leukemia (CML) in early chronic phase (ECP) are tyrosine kinase inhibitors (TKIs) as single agents. Nilotinib (NIL) is a 2nd generation TKI with superior efficacy to imatinib (IM) approved as initial treatment of CML in many countries. A proportion of CML patients treated with single TKIs as monotherapy develops primary or secondary resistance. The use of more than one TKI may decrease the frequency of drug-resistance. **Aims.** To evaluate the response and the outcome of ECP Ph+ CML patients treated with the sequential administration of NIL and IM. **Methods.** A phase 2 study was conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00769327). NIL was administered first because of faster therapeutic effect. Schedule: alternating administration every 3 months of NIL 400 mg twice daily and IM 400 mg daily; the 3-month rotation schedule was respected, irrespectively of temporary discontinuations. In case of toxicity, the patient remained in study, continuing the better tolerated drug alone. Definitions: complete cytogenetic response (CCgR) was defined as the absence of Ph+ metaphases over at least 20 metaphases examined by conventional banding analysis or <1% BCR-ABL+ nuclei over 200 nuclei examined by I-FISH; major molecular response (MMR) was defined as BCR-ABL <0,1%^{IS}; failures were defined according to 2009 ELN criteria; events included death, failure and permanent discontinuation of both drug for any reason, whichever came first. All the calculations were performed according to the intention-to-treat principle. **Results.** 123 patients have been enrolled. Median age was 56 years (range 18-84); 33% low, 45% intermediate and 22% high Sokal score; median follow-up was 27 months (minimum observation: 24 months). CCgR rates were: 72%, 79% and 75% at 3, 6 and 12 months, respectively; the cumulative CCgR rate by 12 months was 87%. MMR rates at 3, 6 and 12 months were 58%, 63% and 65%, respectively; the cumulative MMR rate by 12 months was 82%. The median time to CCgR and MMR was 3 months. At the last contact, 84% of patients were still on treatment with the study drugs, 61% with the alternating schedule, 9% on NIL alone and 13% on IM alone. The overall survival was 94%, the progression-free survival was 93%, the failure-free survival was 87% and the event-free survival 79%. The events leading to study discontinuation were: treatment failure 11%, adverse events 3%, death in chronic phase 2%, second neoplasia 1%, protocol violation 2%. Seven patients progressed to advanced phase: 3 cases with early transformation (within 6 months) developed BCR-ABL kinase domain (KD) mutations (T315 in 2 patients and Y253 in 1 patient). Four patients progressed later on (10 to 24 months) without mutations. **Conclusions.** The response rates and the short-term outcome with the sequential administration of NIL and IM are in the range of the results reported in studies of 2nd generation TKIs as single agents. A longer follow-up is required to assess the effect of this alternating schedule on the occurrence of BCR-ABL KD mutations.**Acknowledgements:** European LeukemiaNet, COFIN, Bologna University, BolognAIL

0586

NILOTINIB INDUCES DEEPER MOLECULAR RESPONSES VS CONTINUED IMATINIB IN PATIENTS WITH PH+ CHRONIC MYELOID LEUKEMIA (CML) WITH DETECTABLE DISEASE AFTER = 2 YEARS ON IMATINIB: ENESTCMR 12-MONTH RESULTS

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Background. The superiority of frontline nilotinib over imatinib in inducing deeper molecular responses in Ph+ chronic phase CML (CML-CP) has been demonstrated in the Evaluating Nilotinib Efficacy and Safety in Clinical Trials"'Newly Diagnosed Patients (ENESTnd) study. These deeper molecular responses observed with nilotinib may increase patients' access to tyrosine kinase inhibitor discontinuation studies in the near future. **Aims.** The ENESTcmr study is evaluating the potential benefit of switching patients with persistent residual disease on long-term imatinib therapy to nilotinib. We report herein the results with a minimum patient follow-up of 12 months. **Methods.** Patients with Ph+ CML-CP who achieved a complete cytogenetic response but had detectable BCR-ABL transcripts by real-time quantitative polymerase chain reaction (RQ-PCR) after ≥ 2 years on imatinib were eligible (N = 207). Patients were randomized 1:1 to receive nilotinib 400 mg BID (n = 104) or continue their imatinib dose (400 or 600 mg QD [n = 103]). The primary endpoint was confirmed complete molecular response (CMR; undetectable BCR-ABL by RQ-PCR with a sensitivity of ≥ 4.5 logs in 2 consecutive samples) by 12 months.

Table 1.

	Nilotinib 400 mg BID (n = 104)	Imatinib 400 or 600 mg QD (n = 103)	*P* Value
CMR by 12 months (ITT population), %			
Confirmed CMR	13	6	.108[a]
CMR	23	11	.02[a]
Molecular response by 12 months (in patients without the response of interest at baseline), %			
	Nilotinib 400 mg BID	Imatinib 400 or 600 mg QD	*P* Value
MMR	n = 24 75	n = 28 36	.006[b]
MR^4	n = 74 49	n = 78 26	.006[b]
$MR^{4.5}$	n = 94 33	n = 91 16	.008[b]
CMR	n = 101 21	n = 100 10	.03[b]
$MR^{4.5}$ by 12 months (in patients with < MR^4 at baseline), %			
$MR^{4.5}$	n = 74 23	n = 78 10	.04[b]
Median time to response, months			
	Nilotinib 400 mg BID (n = 104)	Imatinib 400 or 600 mg QD (n = 103)	*P* Value
MR^4	6	12	.0008[b]
CMR	12	NR	.005[b]
Median BCR-ABL ratio over time, %			
	Nilotinib 400 mg BID (n = 104)	Imatinib 400 or 600 mg QD (n = 103)	
At baseline	0.0234	0.0311	—
At 3 months	0.0152	0.0258	—
At 6 months	0.0094	0.0298	—
At 9 months	0.0070	0.0205	—
At 12 months	0.0065	0.0220	—

ITT, intent to treat; NR, not reached.
[a]Stratified Cochran-Mantel-Haenszel test.
[b]Stratified log-rank test.

Results. Switch to nilotinib resulted in faster and higher rates of molecular response compared with remaining on imatinib. Rate of confirmed CMR by 12 months was higher in the nilotinib arm than in the imatinib arm (13% vs 6%), and CMR by 12 months was significantly higher on nilotinib vs imatinib (23% vs 11%; *P* = .02) (Table). Nilotinib induced superior rates of all levels of molecular response (MMR, MR^4, $MR^{4.5}$, and CMR) in patients without the response of interest at baseline and was associated with significantly shorter time to response than imatinib (Table 1). Among those not in MR^4 or better at baseline, significantly more patients in the nilotinib arm than in the imatinib arm achieved $MR^{4.5}$ by 12 months (23% vs 10%; *P* = .04). Furthermore, patients treated with nilotinib experienced a 0.5-log reduction in median BCR-ABL level by 12 months, whereas imatinib-treated patients had minimal evidence of improvement in molecular response (Table 1). With ≥ 12 months' follow-up, 84% of patients remained on nilotinib and 96% on imatinib. The most common reason for discontinuation was adverse events (AEs). More (9%) patients on the nilotinib arm discontinued because of AEs vs imatinib (1%), likely reflective of the fact that 80% of patients received prior imatinib for ≥ 3 yrs and patients on the imatinib arm remained on this drug. Nilotinib and imatinib were relatively well tolerated, with a safety profile consistent with prior studies. **Conclusions.** Patients with ongoing detectable BCR-ABL transcripts who were switched to nilotinib experienced faster, deeper molecular responses compared with those remaining on imatinib. These results suggest that patients with CML with residual disease on long-term imatinib therapy are likely to benefit from switching to nilotinib, especially those without an MMR on imatinib. Further follow-up is needed, and the study is ongoing.

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BOSUTINIB VERSUS IMATINIB IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA: 30-MONTH UPDATE OF THE BELA TRIAL

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Background. The BELA study compared the efficacy and safety of bosutinib, a dual Src/Abl kinase inhibitor, with imatinib in newly diagnosed chronic phase (CP) chronic myeloid leukemia (CML). **Aims.** The current analysis reports data after ≥24 months of follow-up; updated data including ≥30 months of follow-up will be presented. **Methods.** A total of 502 patients with newly diagnosed CP CML were randomized to bosutinib 500 mg/day or imatinib 400 mg/day and stratified by Sokal risk group and geographic region. Efficacy analyses included all randomized patients (ITT population: bosutinib, n = 250; imatinib, n = 252) or treated patients without major protocol violations who had adequate baseline and either ≥1 post-baseline assessment or disease progression/death prior to the first post-baseline assessment (evaluable population: bosutinib, n = 220; imatinib, n = 241); safety analyses included all treated patients (safety population: bosutinib, n = 248; imatinib, n = 251). **Results.** The median treatment duration was 27.5 months in both cohorts; 63% of bosutinib patients and 71% of imatinib patients were still receiving treatment at the time of this analysis. The primary reason for bosutinib discontinuation was a treatment-emergent adverse event (TEAE; 24% vs 7% with imatinib), while the primary reason for imatinib discontinuation was disease progression (13% vs 4% with bosutinib). Cumulative complete cytogenetic response rates by 24 months were 79% for bosutinib and 80% for imatinib in the ITT population, and 87% versus 81% in the evaluable population (*P* = 0.046). Cumulative major molecular response (MMR) rates by 24 months were 59% for bosutinib (16% complete molecular response [CMR], 4.0-log sensitivity) and 49% for imatinib (12% CMR) in the ITT population (*P* = 0.019). In the evaluable population, cumulative MMR rates by 24 months were 65% for bosutinib versus 51% for imatinib (*P* = 0.002). On-treatment transformation to accelerated/blast phase occurred in 4 (2%) bosutinib patients and 14 (6%) imatinib patients. Kaplan-Meier estimates of event-free survival at 24 months were 92% for bosutinib and 87% for imatinib. Deaths were reported for 7 bosutinib patients (6 due to CML progression) and 13 imatinib patients (10 due to CML progression); 24-month Kaplan-Meier overall survival estimates were 97% for bosutinib and 95% for imatinib. Bosutinib was associ-

ated with higher incidences of gastrointestinal events compared with imatinib (Table); gastrointestinal events were typically transient (eg, median duration of a diarrhea event: 3.0 days for bosutinib vs 5.5 days for imatinib). However, bosutinib was associated lower incidences versus imatinib of musculoskeletal events and edema. The most common grade ≥3 TEAEs with bosutinib were diarrhea, vomiting, and rash (Table 1). Common grade ≥3 laboratory abnormalities with bosutinib or imatinib were neutropenia (10% vs 24%), thrombocytopenia (14% vs 15%), elevated alanine aminotransferase (23% vs 4%), and hypophosphatemia (6% vs 20%). **Conclusions.** Bosutinib was effective for treatment of newly diagnosed CP CML, with higher rates of MMR and CMR than imatinib. With continued follow-up both on-treatment transformation to accelerated/blast phase and overall survival continue to favor bosutinib versus imatinib. Additionally, bosutinib and imatinib had distinct toxicity profiles.

Table 1.

	Bosutinib (n = 248)		Imatinib (n = 251)	
Adverse event, n (%)	All grades	Grade 3/4	All grades	Grade 3/4
Diarrhea	173 (70)	29 (12)	62 (25)	2 (1)
Vomiting	80 (32)	8 (3)	39 (16)	0
Nausea	80 (32)	2 (1)	91 (36)	1 (<1)
Rash	59 (24)	4 (2)	47 (19)	2 (1)
Pyrexia	45 (18)	3 (1)	31 (12)	3 (1)
Upper abdominal pain	35 (14)	0	18 (7)	0
Abdominal pain	33 (13)	3 (1)	17 (7)	1 (<1)
Headache	32 (13)	2 (1)	28 (11)	0
Fatigue	32 (13)	3 (1)	34 (14)	2 (1)
Upper respiratory tract infection	29 (12)	0	20 (8)	0
Cough	23 (9)	0	27 (11)	0
Arthralgia	18 (7)	0	31 (12)	1 (<1)
Myalgia	14 (6)	0	30 (12)	2 (1)
Muscle cramps	11 (4)	0	56 (22)	0
Bone pain	10 (4)	0	26 (10)	2 (1)
Peripheral edema	9 (4)	0	28 (11)	0
Periorbital edema	3 (1)	0	37 (15)	0

Multiple myeloma - Translational research

0588

HIGH CNA LEVEL AND OVER-EXPRESSION OF GENES INVOLVED IN RESPONSE MECHANISMS TO GENOTOXIC STRESS CHARACTERIZE NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS CARRYING AMPLIFIED MDM4 AND/OR DELETED TP53

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Background. The p53 tumor suppressor pathway is tightly kept in check, or completely silenced in cancer cells. A potent inhibitor of p53 is MDM4, which is critical for control of p53 activity during response to stress and is often amplified in several types of tumors. Both *TP53* mutations and del(17p) are infrequently detected in newly diagnosed MM; nevertheless they have been strongly related to patients (pts) survival. Recently, the adverse prognostic impact of chr.1q amplification has been reported in MM. **Aims.** To investigate the prognostic and biological role of del(17p) and/or amp(1q) in newly diagnosed MM pts treated with bortezomib-thalidomide-dexamethasone (VTD) as induction therapy prior to, and consolidation after, double autologous stem-cell transplantation (ASCT). **Methods.** Eighty-eight pts treated with VTD incorporated into double ASCT were analyzed by means of gene expression profile (Affymetrix U133 Plus2.0 array) and unpaired analysis of copy number alterations (CNA) (Affymetrix 6.0 SNP array). **Results.** Thirty out of 88 pts (34%) carried a minimal amplification region of 1,1 Mb on chr.1q, which harbors *MDM4*. Nine out of 88 pts (10,2%) carried a minimal deletion region of 482 Kb on chr.17, which harbors *TP53*. Pts were stratified into two subgroups according to the presence of amplified *MDM4* and/or deleted TP*53* (group A, 36 pts, or 41%) or the absence of both these abnormalities (group B, 52 pts, or 59%). Baseline clinical characteristics were homogeneous, except for a higher rate of IgA isotype in group A.Rates of best complete or near complete response were 81% and 63% in group A and B respectively, with time-to-best-response of 7.7 months (group A) vs. 12.7 months (group B) (p=0.04). Rates of relapse or progression were 58% and 27% for group A and B respectively (p=0.004), with a median progression-free survival of 39.6 months and not reached for pts of group A and B respectively (p=0.04).The average number of aberrations per group was higher in group A as compared with group B (191 vs. 117 CNAs, p =0.03). A comparison of expression profiles of the two groups of pts highlighted an overall deregulation of genes involved in response mechanisms to genotoxic stress, i.e damage sensor genes (*ATM, RAD21*), damage signal mediator genes (*CHK1, MSH2, MSH5*), genes involved in regulation of cell proliferation (*CDKN2A*, *CDC14a*) and anti-apoptotic genes (*CASP6*, *BCL6*, *TP63*) (one-way ANOVA, p <0,01). Finally, group A significantly over-express the transcription factor *YY1*, which is known to interact with p53, thus inhibiting its transcriptional activity. **Conclusions.** Pts carrying amplified *MDM4* and/or deleted *TP53* showed a significantly higher number of CNAs and the significant overexpression of genes involved in response mechanisms to genotoxic stress, as compared to pts lacking these chromosomal aberrations. This might account for the worse outcome of these group of pts. Amplification of *MDM4* locus and over-expression of *YY1* might contribute to maintain p53 in an OFF state by indirect mechanisms. Additional data on the prognostic role of both direct and indirect control of p53 will be presented during the meeting.*Supported by: Ateneo RFO grants (M.C.) BolognAIL*.

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CHROMOSOME 1 ABNORMALITIES PREDICT POOR OVERALL SURVIVAL IN ELDERLY NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS ENROLLED IN THE GIMEMA-MM-03-05 RANDOMIZED CONTROLLED TRIAL

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Background. Chromosome 1 abnormalities (1p deletion and 1q amplification) have been recently analyzed in MM by several Authors. However, no uniform consensus has been obtained, concerning the impact of these abnormalities on clinical outcome. Moreover, their prognostic impact have never been previously evaluated in a huge cohort of elderly newly diagnosed MM patients, but only either in relapsed/refractory or younger patients, eligible for autologous stem cell transplantation. **Aims.** To evaluate the prognostic impact of chromosome 1 abnormalities in 511 newly diagnosed MM patients, older than 65 years, randomly assigned to receive either Bortezomib-Melphalan-Prednisone-Thalidomide followed by maintenance with Bortezomib-Thalidomide (VMPT-VT, N=254) or Bortezomib-Melphalan-Prednisone (VMP, N=257). **Methods.** Samples were suitable for FISH analysis in 336/511 patients (66%). FISH was performed on bone marrow plasma cells (BMPC) purified using anti-CD138-coated magnetic beads. Nuclei from fixed PC were prepared for interphase FISH using standard **Methods.** DNA probes were used to detect 13q14, 1p36, and 17p13.1 deletions, 1qter amplification; t(4;14)(p16;q32), t(14;16)(q32;q23), t(11;14)(q13;q32). **Results.** Frequency of deletion of chromosome 13q14 and 17p13.1 and IgH translocations, and the absence of significant prognostic relevance on PFS have been previously reported by Palumbo et al (JCO 2010; 28 (34): 5101-09). Chromosome 1 abnormalities were evaluated in 278 unselected patients. In the whole cohort of patients, 1q amplification (amp1q) (3 or more copies) was observed in 130 patients (47%) and 1p deletion (del1p) was present in 24 patients (8.6%). Median follow-up was 42 months (range 1-69). The overall median PFS was 32 months. Median PFS was 29.7 months in amp1q-positive and 34.1 in amp1q-negative (p=0.219), while it was 24.6 months in del1p-positive and 33.2 in del1p-negative group (p=0.03). In all the patients median OS was not reached: the 4-year OS was 74%. The 4-year OS was 68.5% in amp1q-positive vs 78.9% in amp1q-negative group, (p=0.04) and it was 58.3% in del1p-positive vs 75.5% in del1p-negative group (p=0.02). Interestingly, in patients carrying at least one aberration of chromosome 1 (amp1q and/or del1p), the shorter OS was even more significant (p=0.005) (Figure 1). In a multivariate Cox regression model, independent predictors of shorter OS were chromosome 1 aberrations (HR, 1.69; 95% CI, 1.00 to 2.85; p=0.05), ISS stage II vs I (HR, 2.37; 95% CI, 1.09 to 5.14; p=0.03) and ISS stage III vs I (HR, 2.72; 95% CI, 1.20 to 6.18; p=0.02), regardless of treatment.**Conclusions** Our results suggest that chromosome 1 abnormalities are an independent poor prognostic factor for OS in MM patients treated with novel Bortezomib-based regimens. A longer follow-up is needed to confirm the significant relevance of these results.

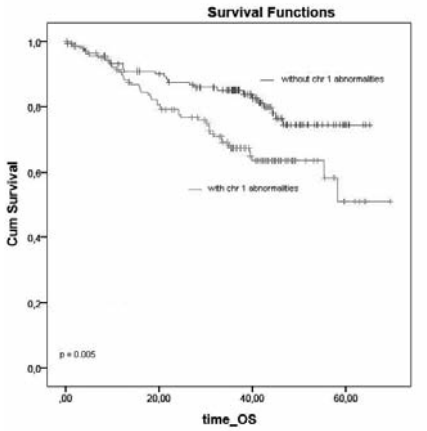

Figure 1. Kaplan-Meier curve for the OS according to chromosome 1 abnormalities.

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CLINICAL IMPLICATION OF CENTROSOME AMPLIFICATION AND EXPRESSION OF CENTROSOMAL FUNCTIONAL GENES IN MM

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Background. Multiple myeloma (MM) is an incurable plasma cell malignancy, a heterogeneous disorder with incompletely understood molecular defects and variable clinical manifestations. Genome of malignant plasma cells (PC) is extremely unstable, characterized by a combination of complex structural and numerical abnormalities. Centrosome amplification (CA) is supposed to be one of mechanisms leading to chrosomal instability. Also, CA is associated with deregulation of cell cycle, mitosis, DNA repair and proliferation. As CA is common in all plasma cell stages including MGUS, it is therefore an early event in MM and can serve as a source of genomic instability. **Aims.** The objective of our study was to evaluate clinical impact of CA as well as its association with changes in expression of genes involved in centrosome structure/function in MM. **Methods.** A total of 216 patients were evaluated for this study. Patients' baseline characteristics were as follows: males/females (122/94), median age of 67.5 years (range 40-85 years). Newly diagnosed (123/216) and relapsed (93/216) patients were included in this study; most of them had advanced stage of MM (D-S II/III 84% [181/216]; ISS II/III 60% [129/216]). CD138+ cells were separated by MACS. Immunofluorescent labeling of centrin was used for evaluation of centrosome amplification (CA) in PCs. Interphase FISH with cytoplasmic immunoglobulin light chain staining (clg FISH) and qRT-PCR (113/216) were performed on PCs. **Results.** Based on immunofluorescent staining results, all newly diagnosed patients were divided to CA positive (40%) and CA negative (60%). MM related early death in two years after diagnosis was significantly higher in CA negative group 19% (6/32) vs 0% (0/29) in CA positive (*P*=0.032). Also, worse OS was indicated in CA negative patients (44/73) in comparison with CA positive patients (29/73) (*P*=0.029). Group of newly diagnosed MM patients showed significant differences in relative quantification coefficient R of the following genes: *AURKB*, *PLK4* and *TUBG1* between CA positive (n=13) and CA negative (n=18) groups of patients (p<0.05).Total of 85% of qRT-PCR samples (96/113) were available for further analyses. Gene expression in newly diagnosed (61/96) and relapsed (35/96) patients showed significant changes of the following (A*URKA, AURKB, CCNB1, CCNB2, CETN2, HMMR, PLK4, PCNT* and *TACC3.* All mentioned genes were upregulated in PC population of relapsed patients (p<0.05).Expression of genes connected with centrosome formation and function was significantly different between HY (25/54) and non-HY (29/54) myeloma patients. *AURKA* was upregulated in non-HY group, while *AURKB* and *PLK4* were upregulated in HY. **Conclusions.** Considering revealed clinical and gene expression heterogeneity between CA negative and CA positive patients, there is a possibility to characterize them as notable event in multiple myeloma pathogenesis. Centrosome abnormality as well as aneuploidy is spatially and timely tightly linked to the cell cycle disruption; CA triggers spindle checkpoints, leading to mitotic catastrophe and accumulation of genomic abnormalities during disease progression in MM. **Acknowledgments.** This work was supported by grants: NT11154, NT12130, MSM0021622434 and by project GAP304/10/1395.

0591

COMPLETE RESPONSE IS A STRONGER PREDICTOR OF SURVIVAL THAN CYTOGENETIC PROFILE AND INTERNATIONAL STAGING SYSTEM STAGE IN ELDERLY MYELOMA PATIENTS TREATED WITH BORTEZOMIB: ANALYSIS OF 771 PATIENTS

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Background. The International Staging System (ISS) and the cytogenetic profile are the most relevant disease-related prognostic factors. The benefit of complete response (CR) in patients with standard or high-risk disease is still a matter of debate. **Aims.** The primary objective of our study was to compare overall survival (OS) and progression-free survival (PFS) of newly diagnosed elderly myeloma patients who achieved CR with those who achieved very good partial response (VGPR) or partial response (PR) after a bortezomib-based regimen. We tested the hypothesis that benefit of CR could vary in different subsets of patients defined according to ISS stage and cytogenetic profile. **Methods.** Newly diagnosed patients included in the GIMEMA-MM-03-05 and the GEM05MAS65 trials were analysed. Details on treatment regimens and results of these studies have previously been reported. Data were pooled together and stratified according to best response achieved. Univariate and multivariate analyses of OS and PFS were performed. Response was treated as time-dependent variable. Landmark analysis with landmark point at 12 months was performed. **Results.** A total of 771 patients were retrospectively analysed. Best response was available in 751 patients: CR was reported in 267 (36%), VGPR in 141 (19%), PR in 242 (32%). Baseline demographics and disease characteristics, including median age, ISS and FISH abnormalities [presence of t(4;14) or t(14;16) or del17p] were similar in patients who obtained CR, VGPR, PR.After a median follow-up of 3 years, the 3-year OS and PFS for all patients were 75% and 30%, respectively. The 3-year OS was 94% in CR patients compared to 86% in VGPR patients (HR 0.30, 95%CI 0.16-0.52, P<0.001). The 3-year PFS was 68% in CR patients compared to 28% in VGPR patients (HR 0.18, 95%CI 0.12-0.28, P<0.001). Subgroup analyses showed that 3-year OS was 86% in patients with ISS stage I, 77% in patients with stage II (HR 0.68, 95%CI 0.46-1.02, P=0.064) and 67% in patients with stage III (HR 0.49, 95%CI 0.32-0.75, P=0.001). In CR patients, 3-year OS was 98% in patients with ISS stage I, 94% in patients with stage II (HR 0.66, 95%CI 0.23-1.89, P=0.434) and 93% in patients with stage III (HR 0.47, 95%CI 0.47-1.39, P=0.171). Similarly, 3-year OS was 76% in patients with standard-risk compared to 65% in patients with high-risk cytogenetic profile (HR 0.65, 95%CI 0.47-0.91, P=0.013), but in CR patients 3-year OS was 94% and 90% in the standard and high-risk group, respectively (HR 0.66, 95%CI 0.29-1.50, P=0.317). In multivariate analyses the achievement of CR was the dominant factor associated with significantly longer OS as compared with VGPR (HR 0.24, 95%CI 0.10-0.55, P<0.001). **Conclusions.** Achievement of CR is a stronger predictor of survival than ISS and cytogenetic profile in patients treated with bortezomib.

0592

ARE WE MAKING PROGRESS? SURVIVAL IN PLASMA CELL MALIGNANCIES IN THE ERA OF NOVEL TREATMENTS A POPULATION BASED STUDY OF 17.790 PATIENTS IN THE NETHERLANDS

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Background. Over the last two decades, the use of High-dose Melphalan (HDM) and the development of novel agents like thalidomide, lenalidomide and bortezomib has changed the management of patients with plasma cell malignancies (PCM) dramatically and extended overall survival.Most results on survival improvement have been generated in randomized clinical trials. However, data from trials refer to a selected subgroup of patients that does not necessarily correspond to the general patient population.AimWe retracted data available from the Dutch Cancer Registry from 1989-2009 in order to get insight into recent and long term trends in survival in unselected patients treated with conventional and novel agents.MethodWe included all adult patients with plasma cell malignancies (i.e. multiple myeloma (MM), plasma cell leukaemia (PCL) and Plasmacytomas; both solitary bone (SP) and extramedullary (EP)), newly diagnosed during 1989-2009 in the Netherlands (n=17.790) We categorized patients into 4 treatment cohorts: 1989-1993:pre-HDM and autologous stem cell transplantation (AutoSCT), 1994-1998:routine use of HDM and ASCT, 1999-2003:thalidomide use for relapsed/refractory patients and 2004-2009: thalidomide use first line. Follow up was completed up to January 1st, 2010. Annual incidence rates were calculated per 100.000 person-year.. Relative survival, which can be interpreted as disease-specific survival within a cancer patient population was derived as the ratio of observed survival of the patients and the expected survival of a comparable age- and sex matched group of the underlying general population. **Results.** 16.822 patients with MM, 111 with PCL, 522 with SP and 335 with EP were diagnosed during 1989-2009. The incidence of PCM increased from 4.3 per100,000 in1989 to4.8 in2009 (estimated annual percentage change (EAPC) 0.4%). Among the subgroups MM and EP there was a trend of increasing incidence, but only for SP and PCL it rose significantly (Table 1). Five-year relative survival significantly improved for all patients with PCM from 30% in 1989-1993 to 39% in 2004-2009(p<0.001). Among the subgroups, significant survival improvement was observed for MM and SP patients(MM: from 28% in 1989-1993 to 37% in 2004-2009; SP: from 59% to 75%, respectively). Most substantial increase in MM survival was observed in patients up to 59 years of age, less pronounced for patient up to 70, while survival did not improve for patients above 70 and even decreased for patients 80+ years. Five-year relative survival decreased slightly for all EP patients from 84% to 70%, although not significant(p=0.44). For PCL patients, survival remains very poor with 1-year relative survival about 40%. However, patients below 65 had a better survival than patients aged 65 and over (in 1989-2009 59% versus 24%).ConclusionAccording to our population based study the incidence of PCM increased during the last two decades. Survival decreased for all patients with EP, remained stable for Patients with PCL but improved significantly for patients with SP and patients with MM up to 70 years. Survival improvement is observed after high-dose therapy and AutoSCT became treatment of choice for younger patients and thalidomide use for the elderly.

Table: age-standardized incidence rates of plasma cell malignancies by subgroup and calander period of diagnosis and estimated annual percentage changes (EAPC)

	age standardized incidence rates			
year of incidence	multiple myeloma	plasma cell leukemia	solitary plasmacytoma	extramedullary plasmacytoma
1989-1993	4.4	0.02	0.14	0.07
1994-1998	4.5	0.02	0.13	0.08
1999-2003	4.4	0.04	0.14	0.08
2004-2009	4.5	0.04	0.19	0.09
EAPC 1989-2009	0.25	5.95	2.02	1.80
(95% ci)	(-0.03, 0.53)	(2.41, 9.50)	(0.25, 3.79)	(-0.50, 4.09)

Translational advances in myeloproliferative neoplasms

0593

GENETIC ANALYSIS OF PATIENTS WITH LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS REVEALS RECURRENT SRSF2 MUTATIONS WHICH ARE ASSOCIATED WITH ADVERSE OUTCOME

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Background. Myeloproliferative neoplasms (MPNs) are chronic, clonal hematopoietic stem cell disorders, individually carrying a risk of morbidity and mortality, including thrombosis,bleeding, and progressive bone marrow fibrosis.Importantly, a substantial proportion of patients with polycythemia vera (PV), essential thrombocytosis (ET) or primary myelofibrosis (PMF) transform to acute myeloid leukemia (AML).Leukemic transformation (LT) from a pre-existing MPN carries a dismal prognosis.Recently, a series of novel genetic alterations in multiple genes encoding members of the spliceosome including SRSF2 was identified. **Aims**. To determine the frequency of somatic alterations in known myeloid disease alleles in MPN patients with LT, and to identify recurrent somatic mutations in the SRSF2 gene in LT after MPN.

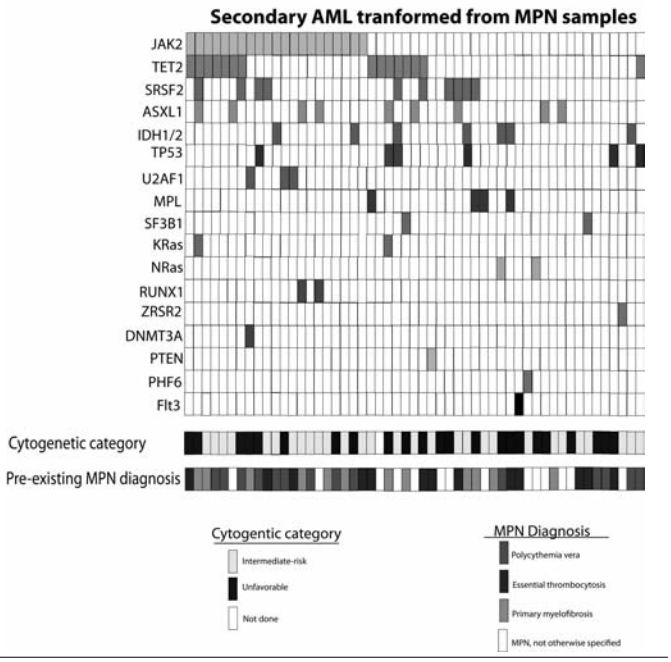

Figure 1. Distribution of mutations amongst patients with acute myeloid leukemia (AML) derived from a myeloproliferative neoplasm (MPN).

Methods. DNA was acquired from 53 patients with AML transformed from an antecendent MPN, 54 patients with *de novo* AML, and 42 patients with AML transformed from MDS.Of the 53 patients with LT of an MPN, 17 samples from the paired chronic phase MPN state were also available for analysis. DNA resequencing of all coding exons of known mutations in JAK2, MPL, IDH1, IDH2, FLT3, c-KIT, K/N/H-Ras, U2AF1, SF3B1, and SRSF2 and full length sequencing of GATA2, RUNX1, EZH2, WT-1, DNMT3a, TP53, TET2, ASXL1, PTEN, and ZRSR2 were performed in all secondary AML and paired MPN samples. Standard statistical methods were used for parameter comparison and survival curves were prepared by the Kaplan-Meier method and compared by the log-rank test. **Results.** Mutations in *JAK2, TET2, SRSF2,* and *ASXL1* are the most commonly mutated genes in this subset of disease. Furthermore, mutations in *SRSF2* are specifically enriched in patients with LT of MPNs compared with patients with *de novo* AML, chronic MPNs, or LT of MDS (p=0.05, two-tailed, Fisher's exact test). In addition, our analysis identified previously unreported somatic mutations in *U2AF1, SRSF2,* and *ZRSR2 in patients with MPN, MDS, and sAML/AML*. Mutational analysis of paired samples from 17 patients with chronic phase MPN who subsequently transformed to AML revealed that mutations in genes outside of the *JAK-STAT* pathway (not in *JAK2* or *MPL*) were significantly enriched in patients at the time of LT compared with the chronic MPN state (average of 1.2 mutations in LT versus 0.6 mutations/sample in chronic phase; p<0.02, Mann-Whitney U test). Correlative univariate analysis of the four most commonly mutated genes revealed that mutations in *SRSF2,* but not in *JAK2, TET2, or ASXL1,* were associated with worsened overall survival in patients with AML derived from MPNs (p=0.0309, HR 2.77 (95% CI 1.10-7.00). The adverse association of *SRSF2* mutations to overall survival was independent of age and cytogenetic risk classification in multivariate analysis (p=0.049, HR 2.11 (95% CI 1.01-4.42). **Summary and Conclusions.** *SRSF2* mutations are associated with adverse outcome in AML after MPN and can be detected in the chronic phase of MPN patients who transform to AML suggests that *SRSF2* mutations may predict for disease progression in MPNs and represent an important event leading to subsequent acute leukemic transformation.

0594

PERTURBATION OF FETAL HEMATOPOIESIS IN A MOUSE MODEL OF DOWN SYNDROME TRANSIENT MYELOPROLIFERATIVE DISORDER

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Background. Fetal and adult hematopoiesis differ by unique gene expression profiles, transcription factor interactions and the presence of specific hematopoietic progenitors. In Down Syndrome (DS), fetal liver hematopoiesis is impaired and characterized by hyper-proliferation of megakaryocytic-erythroid progenitors (MEPs). The ETS transcription factor ERG coded by a gene on chromosome 21 has been one of the candidate oncogenes driving this abnormal proliferation. ERG regulates fetal hematopoietic stem cell maintenance and definitive hematopoiesis. It is expressed in megakaryocyte and erythroid progenitors and is down-regulated upon their differentiation. Its involvement in the DS hematological disorders is supported by a recent study showing that reversing ERG trisomy to functional disomy in the Ts65Dn mouse model corrected the hematological abnormalities in these mice. In About 5% of DS fetuses acquired mutations in *GATA1,* an x-chromosome gene, causing the formation of a shorter protein (GATA1s) lacking GATA1 amino terminal, lead to a congenital transient myeloproliferative disorder (TMD). GATA1s knock-in mice display transient proliferation of immature fetal megakaryocytic progenitors. We previously showed that ERG collaborates with GATA1s to immortalize fetal liver progenitors to generate AMKL. Developmental cooperation between ERG and GATA1 has been also suggested by demonstrating their co-binding to regulatory elements of key hematopoietic transcription factors such as Scl/Tal1. **Aims.** Our goal is to model DS TMD by studying the role of two key regulatory transcription factors, ERG and mutated GATA1, in fetal hematopoiesis and their involvement in generating the hematological abnormalities of DS. **Methods.** To test the hypothesis that ERG and GATA1s cooperate in initiating events of DS TMD, we created transgenic mice expressing the human ERG3 isoform under the Vav promoter, and crossed them with GATA1s Knock-in mice. **Results.** Similar to DS fetuses, ERG Tg mice displayed marked expansion of fetal liver MEPs. This phenotype was enhanced by GATA1s. Both proteins cooperated in enhancement of transient fetal megakarypoiesis. Gene Set Enrichment Analysis (GSEA) demonstrated that the expression profile of fetal liver hematopoietic cells expressing both ERG and GATA1s is significantly enriched with DS-TMD signature genes, suggesting that the biological events occuring in the double transgenic mice are similar to those in the human TMD state.The most profound observation was the almost complete block in primitive erythropoiesis causing the embryonic death of most ERG/GATA1s males. Detailed analysis of primitive erythropoiesis in GATA1s knock-in male embryos (lacking the normal GATA1), revealed markedly decreased formation of BFU-E colonies, dramatic drop in TER119 positive erythroid cells, arrest in erythroid differentiation and increased apoptosis of erythroid cells. These phenotypes were markedly enhanced in double transgenic ERG/GATA1s embryos. The cellular phenotypes were accompanied by decreased expression of erythroid and pro-survival genes such as EPOR, LMO2, KLF1 and Mcl1. **Summary and Conclusions.** We show here that interactions between ERG and GATA1s cause expansion and proliferation of fetal megakaryocytic - erythroid progenitors establishing a fetal TMD like disorder. We further demonstrate a critical role for the amino-terminal domain of GATA1 in primitive erythropoiesis and show that decreased ERG expression is required for terminal erythroid differentiation.

0595

EXTENDING THE SPECTRUM OF PTPN11 GERMLINE MUTATIONS ASSOCIATED WITH JUVENILE MYELOMONOCYTIC LEUKEMIA IN CHILDREN WITH NOONAN SYNDROME

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Background. Noonan syndrome (NS) is an autosomal dominant developmental disorder characterized by the hyperactivation of the RAS-MAPK signaling pathway. In about 40% of cases, a germline mutation in the *PTPN11* gene, encoding the protein tyrosine phosphatase SHP-2, is found. These patients are predisposed to develop a childhood myeloproliferative-myelodysplastic syndrome, the juvenile myelo-monocytic leukemia (JMML). Whereas sporadic JMML is known to be aggressive and to have a poor prognosis, JMML occurring in patients with NS is often considered as a benign and spontaneously resolutive "JMML-like"condition. However, few data are available. **Aims.** We investigated the incidence, genetics and clinical course of myeloproliferative disorders in a large cohort of patients centrally diagnosed with NS in our national reference center. **Patients and Methods.** A cohort of 562 patients carrying a germline mutation of *PTPN11* has been studied. JMML was diagnosed in 21/562 (3.7%) of them (NS-JMML). In 11 other patients (2.0%), hematologic anomalies suggestive of a myeloproliferative-myelodysplastic syndrome have been noted. Cytologic, genetic and clinical features of these 2 groups of patients have been compared with data of 24 patients presenting a *PTPN11*-associated sporadic JMML. **Results.** Hematologic anomalies are found in 32/562 (5.7%) of NS patients and encompass a broad phenotypic spectrum ranging from transient myeloproliferative-myelodysplastic syndrome to JMML. Cytological characteristics of the NS-MPD, NS-JMML and sporadic JMML are similar and *in vitro* endogenous growth of myeloid progenitors is observed in all patients. Conversely, clinical presentation differs between the 3 groups. Median age of onset is earlier in NS patients (neonatal versus 34 months in sporadic JMML). Clinical course of the NS-JMML group may be as aggressive as the sporadic JMML or may resolve progressively, whereas NS-MPD patients have a spontaneously favorable outcome. Among NS patients, 3 have recovered a normal peripheral monocyte count but remain thrombocytopenic throughout follow-up. The one-year overall survival is 100% in NS-MPD group versus only 48% in NS-JMML and 60% in sporadic JMML patients. Notably, boys seem to have a more unfavorable evolution than girls. The D61H mutation of *PTPN11*, never reported in NS, is found in 2 patients with a particularly severe neonatal course. More generally, the spectrum of *PTPN11* mutations is narrower in NS-MPD and NS-JMML patients than in the whole cohort of NS patients, with an increased incidence of mutations in codons 61, 139 and 506. Karyotype and SNP array analysis on 12 NS-JMML patients reveal fewer additional genetic alterations than in sporadic JMML patients. **Conclusions.** Some *PTPN11* mutations are associated with an increased risk of JMML. This genotype-phenotype correlation is confirmed by functional studies on iPS cells derived from 2 of our NS-JMML patients (B Gelb, unpublished). However, the risk of developing MPD or JMML cannot be fully predicted by the underlying *PTPN11* mutation since only a fraction of patients with a given mutation will develop an hematological disease. This suggests that additional factors, possibly gender-related, cooperate with the germline mutation to drive leukemogenesis.

0596

A NOVEL GERMLINE JAK2 MUTATION IN FAMILIAL THROMBOCYTOSIS

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Background. Myeloproliferative neoplasms (MPN), including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) have in most instances a sporadic occurrence. However, familial clustering of MPN has been reported. Common mutations involved in the pathogenesis of MPN such as *JAK2* V617F, *MPL*, *CBL*, and *TET2* mutations are not inherited, but somatically acquired also in familial cases. Germline mutations underlying familial syndromes resembling MPN have been reported. In particular, germline mutations in thrombopoietin (*THPO*) and thrombopoietin receptor (*MPL*) gene have been shown to be responsible for hereditary thrombocytosis.Recently two cases of isolated thrombocytosis associated with novel *JAK2* germline mutations (R564Q and V617I) were reported. **Aims.** In an attempt to identify the germline genetic factors that underlie familial clustering of MPN we applied next generation sequencing to our MPN families. **Methods.** Our cohort of 61 MPN families was analyzed with two strategies. First we applied whole exome sequencing (Illumina instrument) in a subgroup of 16 families with MPN. As this approach resulted in the identification of a novel germline *JAK2* mutation other than V617F in one family, next we sequenced all exons of *JAK2* gene by PCR-based next generation sequencing in the remaining 45 families. Variants were validated by Sanger sequencing. All samples were collected after subjects gave their written informed consent. We used as control population 197 Italian subjects with normal hemogram or a hematological reactive condition. *Results* Exome sequencing resulted in the identification of a novel germline *JAK2* mutation in a pedigree with assumed diagnosis of familial ET. The proband (grandmother, MPC07-116) carried a heterozygous missense mutation (CAT>AAT, chr 9:5073743) of *JAK2* gene, causing a substitution of histidine with asparagine at position 608 (H608N) in granulocytes and T lymphocytes (Figure 1). The mutation was found also in the mother (58), both in granulocytes and in T lymphocytes, thus confirming segregation of the *JAK2* H608N germline mutation with the disease phenotype. The grandson (MPC07-115), a 17-years old male, was a carrier of the *JAK2* H608N germline mutation, as his mother and his grandmother, but he had not yet an overt thrombocytosis. The PCR-based next generation sequencing of the *JAK2* gene in the remaining 45 MPN families did not identify the *JAK2* H608N in any affected member. We did not find H608N in any of the public databases (dbSNP135, 1000 Genomes Project, Exome Variant Server) nor in the Italian control population, thus excluding the suspect of a polymorphism. The novel H608N mutation mapped in exon 14, as the classic V617F mutation. Histidine 608 belongs to the JH2 domain of JAK2 protein, involved in the inhibition of the JH1 kinase activity. **Conclusions.** *JAK2* germline mutations like H608N might account for some cases of familial ET that are indeed cases of hereditary thrombocytosis.

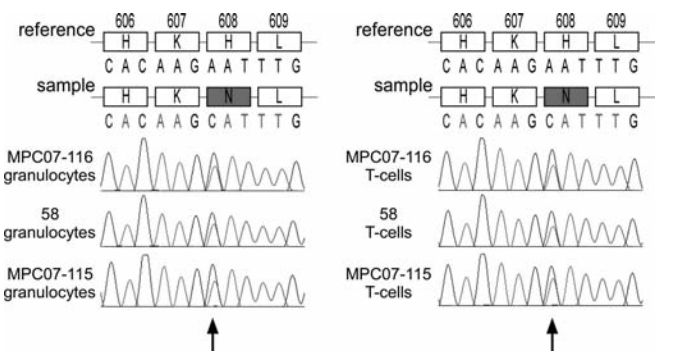

Figure 1. JAK2 H608N germline mutation.

0597

PRELIMINARY SAFETY AND EFFICACY FROM A PHASE II STUDY OF RUXOLITINIB IN PATIENTS WITH PRIMARY AND SECONDARY MYELOFIBROSIS WITH PLATELET COUNTS OF 50-100X10^9/L

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Background. Dysregulation of the JAK-STAT pathway is a central component in the pathogenesis of myelofibrosis, a myeloproliferative neoplasm characterized by splenomegaly, debilitating symptoms, cytopenias, and shortened survival. Ruxolitinib, a JAK1/JAK2 inhibitor, has demonstrated clinical benefit as therapy for patients with myelofibrosis. **Aims.** To explore the safety and efficacy of ruxolitinib in patients with myelofibrosis and low platelet counts, where clinical data are limited. **Methods.** In this phase II study (NCT01348490), patients with intermediate-1 to high risk myelofibrosis and platelet counts of 50-100x10^9/L started ruxolitinib at 5 mg twice daily (BID). Doses could be increased in 5 mg once daily increments every 4 weeks beginning at week 4. Doses were decreased or held for platelet counts <35x10^9/L or <25x10^9/L, respectively. Assessments included Total Symptom Score (TSS, using the modified Myelofibrosis Symptom Assessment Form v2.0, and comprised of scores from 0=absent to 10=worst imaginable for night sweats, itching, bone/muscle pain, early satiety, abdominal discomfort and pain under left ribs); Patient Global Impression of Change (PGIC, a 7-point scale ranging from "very much improved"to "very much worse"); spleen size by palpation and by MRI (data not yet available); and safety. All patients provided written informed consent. **Results.** At the time of analysis, 23 patients had completed ≥4 weeks on study. At baseline, median platelet count was 65x10^9/L, median spleen length was 16.0 cm, and median TSS was 24.5. According to the Dynamic International Prognostic Scoring System classification, 4% were high risk, 52% intermediate-2 risk, and 44% intermediate-1 risk. At the time of analysis, 40%, 26%, 26%, and 4% of patients were receiving ruxolitinib 5 mg BID, 5 mg AM/10 mg PM, 10 mg BID, and 10 mg AM/15 mg PM, respectively. At week 7, one patient (4%) was decreased to ruxolitinib 5 mg once daily for platelet count <35x10^9/L. At week 4 (all patients on ruxolitinib 5 mg BID), mean TSS improved 14%, and 13% of patients had ≥50% improvement. At week 8 (52% on ruxolitinib 5 mg AM/10 mg PM), mean TSS improved 23%, and 30% of patients had ≥50% improvement. Mean spleen length reductions of 22% (week 4) and 27% (week 8) were observed, and PGIC scores of "much improved"or "very much improved"were reported in 35% (week 4) and 39% (week 8) of patients. There were no Grade 4 thrombocytopenia events, no dose holds for adverse events, and no discontinuations from the study; one packed red blood cell transfusion-dependent patient had Grade 4 anemia. Three patients experienced a total of 4 serious adverse events (fever; hypnagogic dreams; spleen pain, pneumonitis) which resolved while on treatment. **Summary and Conclusions.** Effects on spleen size, PGIC, and TSS, even over the first weeks of ruxolitinib treatment when doses were low, are superior to those observed in the placebo group from the COMFORT-I study. These preliminary findings suggest that a dosing strategy starting with ruxolitinib 5 mg BID with subsequent dose optimization may be efficacious and well tolerated in patients with myelofibrosis who have low platelet counts. *Supported by Incyte Corporation.*

Non-Hodgkin Lymphoma - Biology

0598

SELECTIVITY OF ANTIGEN RECEPTORS IN SPLENIC MARGINAL-ZONE LYMPHOMA: FURTHER EVIDENCE FROM THE IMMUNOGLOBULIN LIGHT CHAIN GENE REPERTOIRE

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We recently demonstrated that over 30% of cases with splenic marginal-zone lymphoma (SMZL) express B cell receptors (BcRs) that utilize a single polymorphic variant of the IGHV1-2 gene (IGHV1-2*04). These IGHV1-2*04 receptors carry distinctive antigen-binding sites and also exhibit a low impact of somatic hypermutation (SHM), albeit with preferential targeting of certain positions of the VH domain. These molecular features argue for selection by (super)antigenic element(s) in the pathogenesis of at least a subset of SMZL; in addition, they indicate heavy chain dominance in the clonogenic IG receptors of SMZL. That notwithstanding, the possibility that immunoglobulin (IG) light chains might also play an important role in SMZL ontogeny needs to be investigated, in view of their relevant role in normal, autoreactive and malignant B-cell clones. In order to address this issue, we systematically explored the IG light chain gene repertoire in 114 productive VJ rearrangements from 108 SMZL cases.Seventeen functional IGKV genes were identified in 83 IGKV-J rearrangements; IGKV3-20 was the most frequent gene (20/83 cases, 24%), followed by IGKV4-1 (13/83, 15.7%) and IGKV1-39, IGKV1-5, IGKV1-8/1D-8 (10 cases each, 12%). Collectively, the aforementioned IGKV genes accounted for 76% of all IGKV-J rearrangements. Fourteen functional IGLV genes were identified in 31 IGLV-J rearrangements; IGLV2-14 predominated by far (10/31, 32%). Accordingly, the IG light chain gene repertoire of SMZL is remarkably biased, in analogy to the corresponding IG heavy chain gene repertoire.Based on the percentage of IGKV/IGLV gene identity to the germline (GI), 21/114 sequences (18.5%) were assigned to a truly unmutated subgroup (100% GI), whereas the remaining sequences (93/114, 81.5%) exhibited some impact of SHM activity, ranging from minimal to pronounced. Sequences with 97-99.9% GI were classified as borderline/minimally mutated (n=61, 53.5%), whereas those with <97% GI as significantly mutated (n=32, 28%). Overall, the distribution of IG light chain sequences with regards to SHM status recapitulated what we observed in SMZL heavy chains, implicating a similar impact of SHM on both heavy and light chains.Focusing on the subgroup of 17 IGHV1-2*04 cases with available IG light chain sequence information (19 productive rearrangements), we obtained clear evidence for biased usage of two light chain genes, namely IGKV3-20*01 (6/19 cases, 32%) and IGKV1-8*01 (5/19, 26%). In these pairs, as for the heavy chain (IGHV1-2*04), also the light chain genes (IGKV3-20*01 or IGKV1-8*01) were borderline/minimally mutated. Interestingly, in the case of IGKV3-20*01 sequences, shared (stereotyped) somatic mutations were identified, with IMGT codons CDR1-37 and FR3-66 emerging as hotspots for recurrent, conservative amino acid changes.In conclusion, the present study indicates a complementary role of light chains in shaping BcR specificity and provides further evidence for the highly selective nature of the IG repertoire in SMZL. The restricted molecular features of the IG light chains in the IGHV1-2*04 subgroup may reflect an antigen-driven immune pathway to lymphoma development and further support our recent claims for the existence of distinct subtypes of SMZL defined by immunogenetic analysis.

0599

ONCOGENETIC PATHWAYS IN FOLLICULAR LYMPHOMA REVEALED BY INTEGRATIVE GENETIC ANALYSES IN SEQUENTIAL BIOPSIES

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Background. Genomic imbalances occurring secondary to the translocation t(14,18) are common in follicular lymphomas (FLs), and some are associated with prognosis. Unveiling of transcriptional programs that are affected by gene dosage may provide new insight to the pathogenesis of FL and clues to new treatment approaches. **Aims.** To identify genes and signaling pathways involved in FL pathogenesis and disease progression.

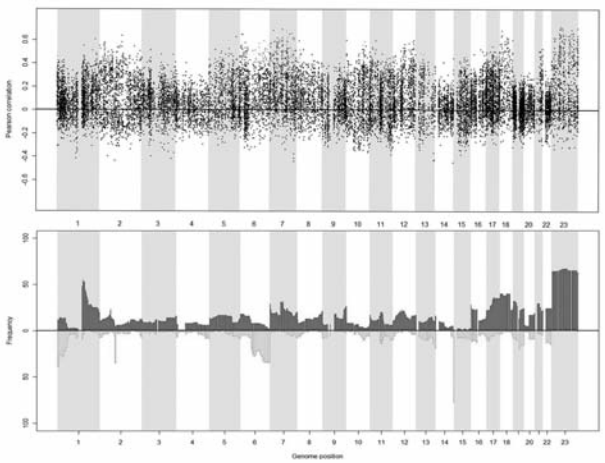

Figure 1 Upper panel: For each of the 21k genes the correlation coeffecients (r) for the relationship between DNA copy number and gene expression levels are plotted according to the respective genomic positions. The 699 genes with r > 0,4 and p < 0.05 are colored red and green according to their location within regions of genomic gains and losses, respectively. **Lower panel:** The frequency of genomic gains (red) and losses (green) in the 100 serial biopsies of FL are plotted according to genomic position. Comparison of the upper and lower panel clearly shows the predominance of positive correlations within frequently aberrant regions.

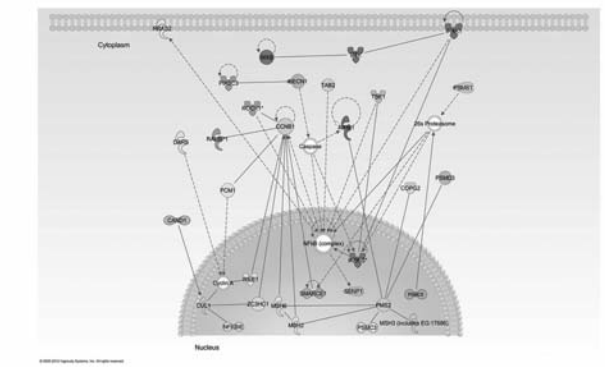

Figure 2: The top interaction network identified by Ingenuity Pathway Analysis (IPA) of the 699 genes showing a highly significant realtionship between DNA copy number and gene expression levels. Thirty-one of the 699 genes participate in the network. Genes colored red and green are located within regions of genomic gains and losses, respectively. The color shading indicates the frequencies of the respective genomic aberrations, with stronger color representing more frequent aberrations.

Methods. The study material consisted of 100 sequential biopsies from 44 patients, including 74 FL grade 1-2, 17 FL grade 3a and 9 FL with various proportions of DLBCL. Twenty-seven cases transformed to DLBCL during the disease course (median observation time 84 months). Seventeen cases never transformed (median observation time 97 months).Whole-genome gene expression profiles were obtained using Affymetrix HG U133 Plus 2.0 Gene Chip in 81 of the sequential biopsies, originating from 41 of the patients. Probes associated with the same gene identifier were averaged to obtain a unique expression value for each gene. The original 51013 probe values were thus reduced to 21485 gene expression values and these were mapped to their respective genomic positions. Whole-genome copy number profiles were obtained for all 100 sequential biopsies using a custom-made BAC/PAC array with a resolution of ~1Mb, and 3091 good-quality probes were used for analysis. The piecewise constant fit (PCF) method was used to segment the raw copy number data and to obtain fitted copy number values (PCF values) for the 3091 probes as well as for the 21k expression probes described above. PCF values above 0.05 were called as gains, and PCF values below 0.05 were called as losses.To robustly assess the effect of copy number on gene expression, two methods were applied. First, the Pearson correlation coefficient between copy number and gene expression in the 81 sequential biopsies was calculated. Second, we grouped the samples for each gene into gain vs. wild type (resp. loss vs. wild type) and used the Student's t-test to compare the expression in the two groups. **Results.** Gene dosage affects the expression level of a large number of genes within aberrant genomic regions in FL (Figure 1). A set of 699 genes (cis-genes) whose expression is profoundly affected by DNA copy number were identified by combining results from the Pearson correlation and the Student's test, applying thresholds of r > 0,4 and a z-score corresponding to p-value < 0.05, respectively. We used Ingenuity Pathway Analyses (IPA) to evaluate the functional associations of these genes. The 699 cis-genes are enriched for genes involved in regulation of cell death, cell cycle control, DNA replication, recombination and repair as well as regulation of cell growth and proliferation. The top interaction network identified by IPA includes genes in the NF-κB pathway, apoptosis signaling, IL-8 signaling and B-cell receptor signaling (Figure 2). **Conclusions and Summary.** We show that genes involved in the NF-κB pathway, apoptosis signaling, IL-8 signaling and B-cell receptor signaling are targeted by genomic imbalances in FL. The relationship of cis-genes to survival and transformation risk will also be discussed.

0600

TET2 LOSS-OF-FUNCTION MUTATIONS ASSOCIATE WITH A DNA HYPERMETHYLATION SIGNATURE IN DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction. The discovery of the functional role of the TET (Ten-Eleven Translocation) family of hydroxylases is a major break through for our understanding of how DNA methylation is deregulated in hematopoietic cancer. DNA demethylation may occur when the TETs convert methylated cytosine to hydroxymethylcytosine. The *TET2* gene is the most commonly mutated gene in myeloid malignancies. Clonal analysis of the hematopoetic stem cell compartment suggests that *TET2* mutations can be early events, and recent investigations showed that the lymphoid lineage was also affected in *Tet2* deficient mice. **Aims.** In the present study we aim to determine the frequency and clinical impact of *TET2* mutations in diffuse large B cell lymphoma (DLBCL) and to evaluate the role of *TET2* mutations on the methylation pattern in DLBCL. **Methods.** Fresh frozen lymphoma biopsies were obtained from 100 newly diagnosed cases of DLBCL and peripheral blood B-lymphocytes were isolated from random, anonymous donors. Clinical data was obtained from the patient files and from the Danish lymphoma registry LYFO. The entire coding sequence and all splice sites of the *TET2* gene (exons 3-11) were scanned for mutations by PCR in combination with denaturing gradient gel electrophoresis (DGGE) and automated sequencing. Global methylation profiling was performed using 450K Infinium arrays (Illumina) and gene expression analysis by Affymetrix arrays. Verification of promoter hypermethylation was done by Methylation-Specific Melting Curve Analysis. **Results.** Here, we show that TET2 is frequently involved in the pathogenesis of DLBCL with 17% carrying missense, nonsense, splice site, and frame shift mutations or deletions. Interestingly, most *TET2* loss-of-function mutations are associated with a hypermethylation signature. Using a stringent statistical analysis, we identified 111 differentially methylated probes in 70 genes all of which were significantly hypermethylated in *TET2*mut/del compared to *TET2*wt cases (p<0.01; t-test). Ingenuity pathway analysis of these differentially methylated genes showed that the majority act in a network involving hematopoietic system development and function, cellular development, cell cycle regulation and cancer. We observed a significant enrichment of the *TET2* signature probes being located at the promoter regions (TSS, 5'UTR and first exons) (73%) and gene bodies (26%). The correlation of methylation patterns with gene expression data is currently being analyzed and will be presented at the meeting. No difference in overall survival and in other clinical parameters were observed between *TET2*mut and *TET2*wt cases. **Summary**. We suggest that *TET2* loss-of-function mutations may lead to disruption of normal TET2 demethylase activity in hematopioetic progenitors and give rise to DNA hypermethylation in a set of genes involved in hematopoietic development and cell cycling. Depending on the secondary molecular changes this may give rise to either myeloid or lymphoid malignancies.

0601

TRANSCRIPTIONAL REGULATION OF CD20 LEVELS IN LYMPHOMA CELLS IS REGULATED BY SRC FAMILY KINASES

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Background. Anti-CD20 monoclonal antibodies have made a breakthrough in the treatment of non-Hodgkin's lymphoma and chronic lymphocytic leukemia. They trigger indirect effector mechanisms of the immune system, namely complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and immunophagocytosis. Although for many years CD20 has been described as a stable antigen, accumulating evidence indicates that CD20 can be modulated at both transcriptional and posttranscriptional levels.

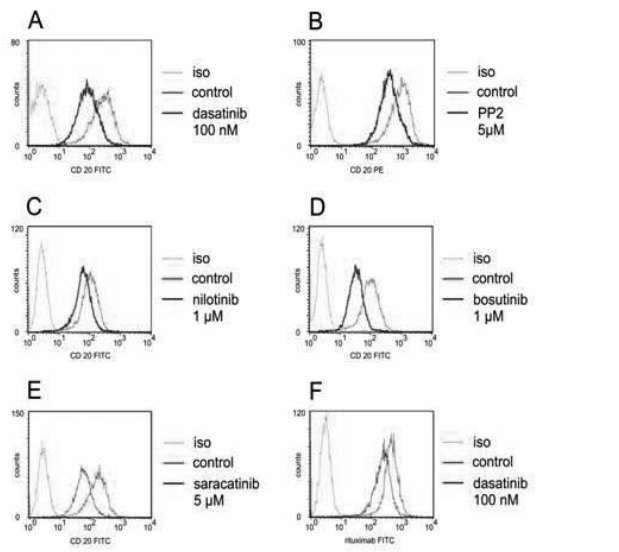

Fig.1 Raji cells, pretreated for 48 hours with various Src family tyrosine kinases inhibitors,were incubated with saturating amount of FITC-conjugated anti-CD20 mAb (**A-E**) or rituximab and secondary FITC- conjugated anti-IgG1 antibody **(F)** for 30 min at room temperature in the dark. Binding of mAb was determined with flow cytometry

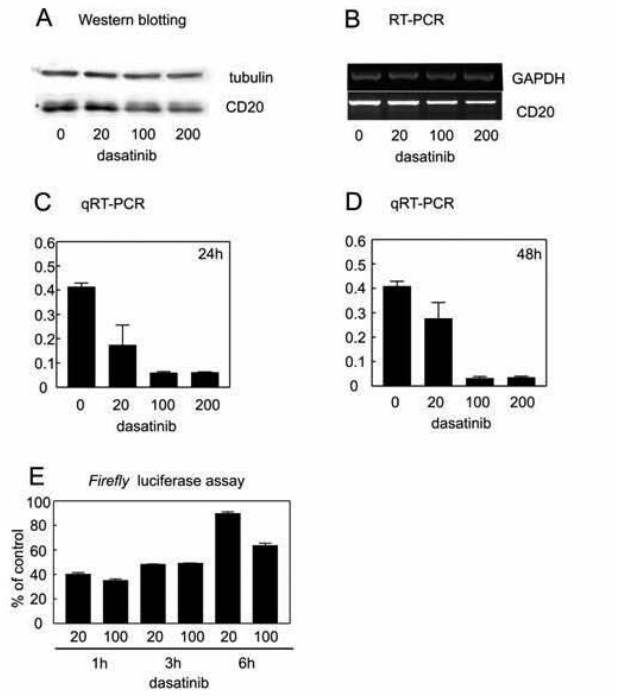

Fig.2 Raji cells were incubated with dasatinib for 48 hours, lysed with RIPA buffer and assayed by Western blotting for total CD 20 and tubulin levels **(A)**.CD20 mRNA levels in Raji cells incubated with increasing doses of dasatinib were assayed by RT-PCR **(B)** or qRT-PCR with specific hydrolysis oligonucleotide probes (**C-D**). Raji cells were stably transduced with CD 20 promoter-luciferase reporter gene construct (pCD20-Luc), incubated for indicated time with dasatinib and assayed for luminescence normalized to protein concentration **(E)**.

It seems that down-regulation of CD20 levels is one of the reasons of tumor resistance to rituximab. Src family kinases (SFKs) including Lyn, Fyn and Lck have been already reported to associate with CD20. However, to the best of our knowledge, the role of SFKs in the regulation of CD20 expression has not been studied so far. **Aims.** The aim of this study was to explore the molecular basis for Src family kinases-dependent regulation of CD20 levels in lymphoma cells. **Methods.** CD20 surface levels and rituximab-mediated CDC (R-CDC) in CD20-positive lymphoma cell lines and primary cells from patients were determined with flow cytometry. Total CD20 protein levels were assayed with Western blotting, the expression of CD20 gene was determined with both RT-PCR and qRT-PCR. The CD20 promoter activity was measured with reporter *Firefly* luciferase assay. Binding of transcription factors to the promoter of *cd20* gene was assayed using chromatin immunoprecipitation. **Results.** Initial experiments showed upon treatment with SFKs inhibitors a significantly reduced binding of anti-CD20 mAb to lymphoma cell lines as well as primary cells isolated from patients. All tested SFKs inhibitors, namely dasatinib, PP2, saracatinib, bosutinib, nilotinib, Btk-, Lck-, Src- and two Syk- inhibitors, as well as shRNA targeting Lyn kinase impaired R-CDC over a dose range of rituximab concentrations (1-100 ug/mL) in Raji cells. Interestingly, in Raji cells incubated for 48h with dasatinib we also observed a dose-dependent reduction of total CD20 protein levels, when assayed by Western blotting. Moreover, a 48-h incubation with dasatinib significantly reduced the transcription of *cd20* gene, as assessed with RT-PCR. To further elucidate the mechanism of transcriptional regulation of CD20 we performed qRT-PCR. A strongly reduced transcription of *cd20* gene was observed in Raji cells over a dose range of dasatinib (20-200 nM) after 24- and 48h- incubation. Additionally, the CD20 promoter activity measured with reporter *Firefly* luciferase assay has been reduced as early as 1 hour after dasatinib treatment. This effect was not observed for truncated CD20 promoter. To elucidate in more detail binding of transcription factors to the promoter of *cd20* gene, a chromatin immunoprecipitation assay was performed. Our early results indicate that dasatinib impairs binding of PU.1 transcription factor to its consensus site within *cd20* promoter in Raji cells. **Summary and Conclusions.** Our studies indicate for the first time that SFKs are involved in the transcriptional regulation of CD20 levels in lymphoma cells. Elucidation of the exact mechanism of this phenomena needs further studies. Results of these experiments will help to understand the biology and regulation of CD20 levels in lymphoma cells.

0602

HIGH THROUGHPUT ANALYSIS OF ANTIGEN RECOGNITION BY THE B CELL RECEPTORS OF MALIGNANT LYMPHOMAS WITH HIGH DENSITY PROTEIN MICROARRAYS

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Background. Shared structural features of the B-cell receptor (BCR) suggest some role of antigen recognition in B-cell non-Hodgkin lymphomas (B-NHL) etiology. The BCRs expressed by CLL and marginal zone lymphoma (MZL) may recognize common autoantigens. Systemic autoimmune diseases are associated with certain B-NHL. Organ-restricted chronic inflammation plays an etiological role in extranodal MZL of MALT type (i.e. H. pylori-associated gastritis, Sjogren's syndrome). Despite this evidence, there has been no systematic and comprehensive assessment of autoantigen recognition by BCR expressed by B-NHL. **Methods.** BCR from 45 B-NHL (13 mantle cell lymphomas, 10 CLL, 5 nodal MZL, 5 diffuse large B-cell lymphomas, 4 follicular lymphomas, 3 myelomas, 2 splenic MZL, 2 lymphoplasmacytic lymphoma) were expressed as recombinant Fab fragments. Binding of lymphoma-derived BCR was tested simultaneously to 8000 human proteins displayed on high-density microarrays. Bound BCR were detected by AlexaFluor labeled antibodies. Z-scores and Z-factors were calculated for each Fab-protein interaction. Positive binding was defined as either Z-score >1.65 and Z-factor >0, or Z-score >1 and Z-factor >0.5 (Zhang et al., 1999). 27 BCR-protein interactions were validated by indirect ELISA. **Results.** 108 BCR-protein interactions involving 48 different proteins were identified. 21 BCR did not bind any protein; 12 BCR recognized one protein. 9 BCR were polyreactive as defined by binding to ≥5 proteins. MZL were classified as polyreactive with very similar recognition patterns, including the autoantigens cardiolipin and Ro-60/SS-A. Unexpectedly, 8 of 9 polyreactive BCR showed evidence of somatic hypermutation (mean: 7.1% range: 3.0-18.6%). The arrays showed a sensitivity and specificity of 99% and 95% respectively with positive correlation between ELISA OD and z-scores ($r=0.74$, $p<0.001$). Although the arrays used here carried the most comprehensive assembly of proteins available, they represent only a fraction of the human proteome. Therefore, homologues to recognized proteins were identified by BLAST and included, among others, the paraneoplastic neuronal autoantigen

Ma1 as being potentially recognized by 4 lymphomas, including a primary CNS lymphoma, and 2 cell wall proteins of pathogenic bacteria. **Conclusions.** This pilot study employing a broad selection of lymphoma types establishes protein microarrays as a novel and valuable platform to study antigen recognition by lymphoma in an unbiased and quantitative fashion, and to deduct a comprehensive pattern of putative antigen stimulation in NHL development. This platform permits definition of recurrent oligo- and polyreactivity patterns of antigen recognition by lymphoma BCR. Unlike in physiological B-cell affinity maturation, somatic hypermutation in B-NHL appears not to result in loss of polyreactivity, which may then continue to provide stimulation of the malignant cells via their BCR. These reactivity patterns appear to operate across various lymphoma entities, suggesting two different components in lymphoma development: A common requirement for BCR-mediated stimulation in the majority of cases, which may occur during various scenarios and may be either specific or follow definable patterns of cross-reactivity, and subsequent malignant transformation of the stimulated cell by genetic alterations. The latter step would be expected to define the lymphoma type through the developmental stage of the cell of origin and the nature of the oncogenic mutation.

Red cells

0603

INTERNATIONAL SURVEY OF T2* CARDIOVASCULAR MAGNETIC RESONANCE IN THALASSEMIA

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Background. Beta thalassaemia major (TM) is a substantial global health issue, with over 25,000 affected children born each year. Accumulation of cardiac iron is the cause of heart failure and early death in many TM patients who depend on regular blood transfusions. Cardiovascular magnetic resonance (CMR) T2* measurement has been shown to provide an accurate, reproducible measurement of cardiac iron and this technique has now been adopted as part of routine management in many countries. A dramatic 71% decrease in deaths has been observed in the UK thalassaemia cohort since the introduction of improved chelation and the routine use of CMR T2*. Whilst cardiac T2* has a strong prognostic value in the UK cohort, little is known about the burden of cardiac iron loading, its effects or the application of CMR T2* across different geographical regions. **Aims.** To gain an understanding of the worldwide use of CMR T2* via an international survey of centres that regularly use T2* to assess its clinical application, the degree of iron loading and relation to clinical outcomes.

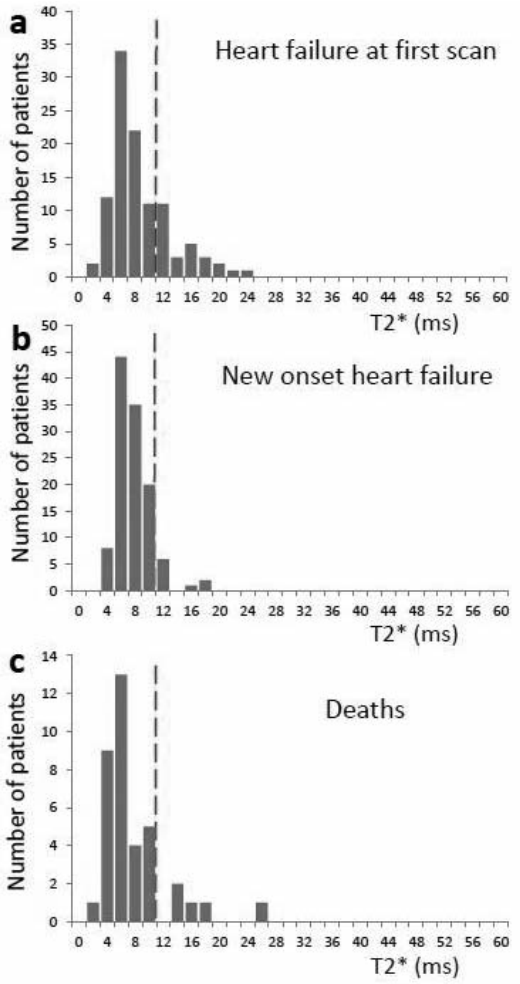

Figure 1. Distribution of T2* values. A: Patients in heart failure at first scan; B: New onset heart failure; C: Deaths.

Methods. A survey was undertaken in 35 worldwide centres of 3,445 patients from Europe, the Middle East, North America, South America, North Africa, Australia and Asia. Anonymised data on myocardial T2* values were analysed in conjunction with clinical outcomes (heart failure and death). For the purposes of this study, cardiac failure was defined as symptoms or signs of cardiac failure associated with objective evidence of ventricular dysfunction at rest (defined as reduced left ventricular ejection fraction <56% measured by CMR). **Results.** Of the total of 3,445 patients, overall 57.7% had no significant iron loading (T2*

>20ms), 22.5% had moderate cardiac iron (10ms < T2* ≤ 20ms) and 19.8% had severe cardiac iron (T2* ≤10ms) at baseline. The prevalence of moderate (T2* <20ms) and severe (T2* <10ms) myocardial iron loading varied significantly between regions with the lowest level being found in patients from Egypt and the highest in South-East Asia (P <0.001). At the time of the first scan, 107 patients (3.5%) had confirmed heart failure, the majority of whom (75.7%) had myocardial T2* <10ms, with 98.1% having T2* <20ms. During follow-up, 116 patients subsequently developed heart failure, and of these, 92.2% had T2* <10ms and 100% had a T2* of <20ms at the time of the first T2* CMR scan. There were 38 deaths during follow-up: at baseline scan, 86.4% had myocardial T2* <10ms, and 97.2% had myocardial T2* <20ms. **Conclusions.** In this well-treated cohort of TM patients from centres across the world who had access to regular transfusion, chelation and T2* CMR, a large proportion (42.3%) had moderate to severe cardiac iron loading. Cardiac T2* values <10ms were strongly associated with the development of cardiac failure and death. There were marked regional differences in the prevalence of cardiac siderosis which may reflect differences in predisposition to cardiac iron loading.

0604

HBA2 LEVELS IN ADULTS ARE INFLUENCED BY TWO DISTINCT GENETIC MECHANISMS

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Background. The switch from embryonic to fetal hemoglobin (HbF, α2γ2) in utero, and from fetal to adult hemoglobin at birth is well documented and achieved by the sequential activation of ε, γ and δ/β genes at the β globin gene (*HBB*) cluster. A change in the expression of hemoglobin genes also takes place in adult erythropoiesis: earlier erythroid progenitors have been shown to produce significant amounts of fetal hemoglobin, while the more mature progenitors contain essentially none. In keeping with the sequential activation of β-like globin genes, δ globin chain synthesis also declines as maturation in erythroid progenitors progresses. Understanding the developmental changes of gene expression at the beta globin locus is not purely of academic interest, since a therapeutic induction of HbF or HbA_2 (α2δ2) production would be of significant clinical benefit for patients with a defect of HbA (α2β2) function or abundance, such as sickle cell disease or β thalassemia. We have previously studied the genetic regulation of fetal hemoglobin persistence in a genome-wide association study (GWAS) in healthy volunteers, and are now extending this approach to the study of HbA_2. **Aims.** To assess the relationship between SNPs and the HbA_2 trait using a quantitative trait GWAS analysis. **Methods.** Our study population is the 'Twins UK' twin registry of healthy Europeans, mostly female adult individuals, with genome-wide single polymorphisms (SNP) data and hemoglobin phenotypes for a primary study group (n=2,322) and a second replication group (n=1,716).

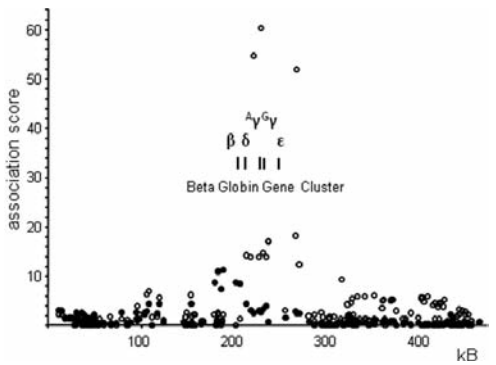

Figure 1. Association with single-nucleotide polymorphisms (SNP) near the beta globin gene cluster on chromosome 11p15.4 with abundance of HbA2.

Results. We found that HbA_2 (as a percentage of total hemoglobin) correlated weakly, but significantly, with the amount of fetal hemoglobin carrying cells (F cells) ($r = 0.14$, $p < 0.01$). This suggests the existence of some common biological process that influences both hemoglobin species. We also found that the same SNP alleles at chromosome 6q23.3 (*HBS1L-MYB*, peak signal rs7775698, $p = 2.51\times10^{-9}$) that are associated with F cells and and red blood cell volume (denoted by the mean cell volume or MCV) also promote HbA_2 levels, again pointing to some common biological factor connected with the erythropoietic maturation process. Interestingly, neither of the other two major HbF loci, *BCL11A* on chromosome 2p, or the HbF-promoting regions within the *HBB* cluster (at the β LCR and the γ globin genes) on chromosome 11p, showed association with HbA_2 levels. Instead, SNPs around the β globin gene itself (clearly separate also from the delta gene) exert a significant influence on HbA_2 levels (peak association rs12793110, $p=5.11\times10^{-12}$) (see Figure 1). In contrast to the *HBS1L-MYB* region on chromosome 6, the HbA_2-boosting alleles at these SNPs do not increase red blood cell MCV. **Conclusions.** Our results suggest that HbA_2 levels in adults are influenced by two distinct genetic mechanisms; one via the kinetics of erythropoiesis, and the other via a competitive process between *HBB* and *HBD* gene activity, mimicking a mild thalassemic effect. The systematic genetic study of specialized hematological traits in healthy volunteers can help to understand the biology of hematopoiesis.

0605

BIOSIMILARS IN THE MANAGEMENT OF ANAEMIA SECONDARY TO CHEMOTHERAPY IN SOLID TUMOURS, LYMPHOMA AND MYELOMA: THE ORHEO STUDY

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Background. Approval of epoetin biosimilars in the EU requires extensive scientific evaluation and stringent regulatory procedures. These range from preclinical and clinical studies to tightly controlled manufacturing processes. Post-marketing pharmacovigilance forms an integral part of the regulatory requirements for biosimilar products. The ORHEO (place of biOsimilaRs in the therapeutic management of anaemia secondary to chemotherapy in HaEmatology and Oncology) study examined the post-marketing efficacy and safety of biosimilar epoetins for the treatment of chemotherapy-induced anaemia in the clinical setting. **Aims.** To evaluate the efficacy and safety of biosimilars of epoetin alfa (EA) for the treatment of chemotherapy-induced anaemia in oncology and haematology in the clinical setting. **Methods.** ORHEO is an observational, non-interventional, longitudinal study performed in multiple centres in France. Patients >18 years with anaemia (haemoglobin [Hb] <11g/dL) secondary to chemotherapy for solid tumours, lymphoma or myeloma and eligible for treatment with EA biosimilar were included. Baseline demographic and disease-related characteristics were recorded at baseline along with anaemia-related data including Hb level, target Hb chosen by the treating clinician, brand and dose of EA biosimilar prescribed, and details of any other concomitant treatments (eg iron). Patients were followed up at 3 and 6 months. Analyses included achievement of target Hb, and Hb response defined as achievement of target Hb without blood transfusions in the preceding 3 weeks and during treatment *or* Hb ≥10 g/dL *or* Hb increase ≥1 g/dL since inclusion. The rate and type of adverse events were also evaluated. **Results.** In this study 2311 patients (mean age 66.5 years) from 232 centres were included. The majority of patients (79.6%) had solid tumours, 13.0% had lymphoma and 7.4% had myeloma. All but one patient received the biosimilar Retacrit (epoetin zeta, median dose 30 000 UI/week). The mean baseline Hb level was 9.6 g/dL, with target levels of 12-13 g/dL for 51% of patients. A total of 2056 and 1664 patients had at least one Hb value at 3 and 6 months, respectively, and Hb response was achieved in 81.6% and 86.5% of patients at these respective timepoints. Of these patients, 35.2% and 28.5% of patients achieved target Hb levels at 3 and 6 months, respectively. Where transfusion data were available, transfusion rates were 9.4% and 5.8% at 3 and 6 months, respectively. Of those patients who discontinued the treatment early, 53.7% at 3 months and 53.7% at 6 months did so due to early achievement of target Hb. Mean time to achievement of target Hb was 80.1 days for the responder population. The rate of thromboembolic events was 2.4% and 1.5% at 3 and 6 months, respectively. **Summary and Conclusions.** Retacrit was effective and well-tolerated in the management of chemotherapy-induced anaemia in patients with solid tumours, lymphoma and myeloma. The vast majority of patients achieved Hb response at 3 and 6 months.

0606

A 48 WEEK PHASE 2 STUDY OF THE SAFETY, TOLERABILITY AND EFFICACY OF FBS0701, A NOVEL IRON CHELATOR FOR TREATMENT OF TRANSFUSIONAL IRON OVERLOAD

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Background. FBS0701 is a novel, orally available iron chelator in clinical development for transfusional iron-overloaded patients. The primary outcome measure of protocol FBS0701-CTP-04 was the effect of a 24 week course of once-daily FBS0701 at two dose levels, 14.5 and 29 mg/kg/d (mpk), on liver iron content assessed by R2 (Ferriscan®) MRI. There was a clear dose response with no safety signals (Neufeld *et al* Blood, 2012. [Epub ahead of print]). Here we present 48 week data from an extension of CTP-04. **Aims.** To assess the safety and efficacy of FBS0701 in adults with transfusional iron overload after treatment for 48 weeks with dose modifications allowed at or after Week 24. **Methods.** Patients who had completed the first 24 weeks of CTP-04, had an LIC ≥2 mg/g dry weight, cardiac T2* ≥10 ms and LVEF ≥55% were eligible to participate. Patients, all with hemoglobinopathies, were dose-adjusted at or after week 24 if there was evidence of iron accumulation: patients randomized to receive 14.5 mpk were increased to 29 mpk; patients randomized to 29 were increased to 36 mpk, the highest permitted dose at that time. Patients were evaluated monthly including history, physical exam, clinical pathology, adverse events (AEs), ECG and LIC by MRI at Week 24 and 48. **Results.** Of 42 patients enrolled in the extension, thirty-nine (93%) completed 48 weeks of treatment; three withdrew for concerns about efficacy. Mean LIC at Baseline was 12.5 (N=42; SEM±1.1) and 13.2 mg/g (N=42; SEM±1) at Week 24. Seventeen (40%) patients had abnormal baseline transaminases and 18 patients (43%) had a history of viral hepatitis. At Week 24, 19 (95%;N=20) patients randomized to 14.5 mpk were dose-adjusted upward, the majority (N=16) to 29 mpk while 14 (64%; N=22) patients randomized to 29 mpk were increased to 36 mpk. FBS0701 was generally well tolerated at all doses. There were no treatment-related SAEs. No AEs showed dose-dependency in frequency or severity. Fewer than 5% had treatment-related nausea, vomiting, abdominal pain, or diarrhea. The most common treatment-related AE was an increase in transaminases (14%, N=6). No patient had a clinically significant increase in serum creatinine (Cr). The average change in LIC from Week 24 to 48 was -0.7 mg/g (N=39; SEM±0.5). **Conclusions.** FBS0701 was well tolerated up to 36 mpk; a maximum tolerated dose was not identified. No safety signals were noted at the higher doses. Treatment-related GI AEs were infrequent and Cr was stable. Transaminases increased in six patients, two of whom acquired HCV simultaneously from a single source as previously reported. Transaminase increases were <2-fold baseline, reversible and restricted to patients with a history of viral hepatitis. FBS0701 was effective at clearing hepatic iron in a majority (67%) of patients dosed at 36 mpk. Higher doses of FBS0701 might be expected to safely induce negative iron balance in a greater proportion of iron overloaded patients.

0607

A NOVEL KLF1 GENE PROMOTER VARIANT (G.-148 G>A) IS ASSOCIATED WITH HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

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Background. Hereditary persistence of fetal hemoglobin (HPFH) is characterized by persistent high levels of fetal hemoglobin (HbF) in adults. Several genetic factors that control HbF levels in adults have already been identified (*HBB*, *HBS1L-MYB*, *BCL11A* genes), while others remain elusive. Recent studies have reported variants within the *KLF1* gene, leading to high levels of HbF in adults. Elevated HbF levels may ameliorate the clinical phenotype of β-thalassemia. **Aims.** The aim of this study was to investigate the properties of a novel g.-148G>A *KLF1* gene promoter variant that was identified in individuals with elevated levels of fetal hemoglobin of Serbian and Greek origin. **Methods.** Mutation screening in the *KLF1*, *HBB, HBG1* and *HBG2* genes was done using PCR and direct resequencing. For the functional analysis, constructs containing the wild type *KLF1* promoter fragment and a *KLF1* promoter fragment bearing the g.-148G>A variant, were transfected into K562 cells and the promoter activity was measured as chloramphenicol acetyltransferase (CAT) activity. For electrophoretic mobility shift assays (EMSA), nuclear extracts from K562 cells and radiolabelled probes corresponding to the wild type (WT) and mutated (MT) *KLF1* sequences were used. In addition, "supershift"assay was performed using Sp1 antibody. **Results.** Three adult patients, presented with high level of HbF and with no mutations in the *HBB*, *HBG1* and *HBG2* genes, were analyzed for the presence of mutations in the *KLF1* gene. Sequencing analysis revealed that the patients were heterozygous for the g.-148G>A variant in the *KLF1* gene promoter. The results of the functional analysis showed the presence of significantly decreased activity (p=0.009) of the *KLF1* promoter bearing the g.-148A variant, compared to the wild type promoter. These results are in concordance with the results of *in silico* analysis, which showed that *KLF1* g.-148G>A promoter variant abolishes binding site of the Sp1 transcription factor. EMSA and "supershift"assays confirmed these data. Namely, addition of Sp1 antibody leads to the formation of a protein complex only in the case of nuclear proteins-KLF1 WT probe interaction, while no effect was observed in the reaction with KLF1 MT probe. **Conclusions.** Our study showed the presence of -148G>A variant within the *KLF1* gene promoter in three patients with elevated HbF level. We propose that this mutation influence *KLF1* gene expression by decreasing *KLF1* promoter activity, which, in turn, affects transcription of the γ-globin genes and, hence, HbF production.

Platelets

0608

MICRORNA REGULATE IMMUNOLOGICAL PATHWAYS IN T-CELLS IN IMMUNE THROMBOCYTOPENIA (ITP)

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Background. ITP is an organ specific autoimmune disease in which T-cells play an important pathophysiologic role. MicroRNA are small non-coding RNA molecules that regulate gene expression. MicroRNA have been implicated in other autoimmune diseases but so far not studied in ITP. **Aims.** To investigate microRNA as a potential regulator of gene expression in T-cells from adult patients with chronic ITP. **Methods.** Blood was obtained from patients with chronic ITP and healthy controls followed by T-cell isolation and RNA preparation. For the genome-wide expression analyses RNA from 9 chronic ITP patients and 10 healthy controls was amplified using the Ovation amplification system V2 (NuGEN Technologies Inc) and hybridized to U133plus2.0 DNA microarrays (Affymetrix). MicroRNA from 9 ITP patients and 9 controls was labeled using the FlashTag Biotin HSR kit (Genisphere) and subsequently hybridized to the microRNA 2.0 microarrays (Affymetrix). Genes with different expression between ITP and controls were classified according to Gene Ontology. The target genes of the differently expressed microRNA were identified using the algorithms TargetScan and Miranda. The impact of the microRNA on gene expression was evaluated using the Kolmogorov-Smirnov test and the target genes from these microRNA were cross referenced against the list of significantly regulated mRNA between ITP patients and controls, identified in the T-cell gene expression analysis. Plasma levels of CXCL13 were analyzed by ELISA. **Results.** We identified 1915 regulated genes and 22 regulated microRNAs with different expression in ITP patients and controls. Seventeen of the 22 regulated microRNA had target genes with an affected expression; 57 of these target genes were associated with the immune system, e.g. T-cell activation and regulation of immunoglobulin production. Two microRNA target genes significantly increased in ITP were CXCL13 and IL-21. CXCL13 has been demonstrated to be central in the development of B-cell follicles and clonal B-cell expansion. IL-21 is the cytokine responsible for Th17 cell differentiation and cytotoxic T-cell responses in chronic infections, as well as B-cell proliferation and immunoglobulin class switch recombination. In line with the mRNA data we could demonstrate increased plasma levels of CXCL13 in ITP compared with controls. In addition, other investigators have previously reported increased plasma levels of IL-21 in ITP. **Conclusions.** Regulated microRNA were significantly associated with both gene and protein expression of molecules in immunological pathways suggesting that microRNA may be important regulatory molecules involved in the loss of tolerance in ITP.

0609

ELTROMBOPAG RAISES PLATELET COUNTS PRIOR TO ANTIVIRAL THERAPY IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION ASSOCIATED WITH THROMBOCYTOPENIA

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Background. Thrombocytopenia (TCP) is common in cirrhotic patients with hepatitis C virus (HCV) infection and is correlated with disease severity (Louie 2011). TCP may impair or prevent initiation of peginterferon alfa (PEG) therapy and necessitate PEG dose reduction or discontinuation, reducing or denying potentially curative treatment options for these patients. Eltrombopag, an oral, nonpeptide, thrombopoietin receptor agonist, increases platelets in patients with TCP due to HCV-related cirrhosis (McHutchison 2007). ENABLE 2 was a phase 3, multicenter, two-part study of eltrombopag for patients with HCV who are ineligible or poor candidates for antiviral therapy due to TCP. **Aims.** To assess safety and efficacy of eltrombopag during the open-label treatment phase (Part 1) of ENABLE 2 and the ability of eltrombopag to enable initiation of antiviral therapy (Part 2). **Methods.** Patients with chronic HCV and a baseline platelet count <75,000/μL received oral eltrombopag (25 mg daily with dose escalations every 2 weeks to a maximum of 100 mg) for up to 9 weeks until platelets reached ≥100,000/μL. Patients achieving these counts were eligible for randomization (2:1) to eltrombopag or placebo at the final dose received in Part 1, in combination with antiviral therapy (PEG-2b plus ribavirin) for up to 48 weeks (Part 2). Patients who failed to achieve platelets ≥100,000/μL following 3 weeks of eltrombopag 100 mg daily did not enter Part 2 and attended scheduled follow-up visits. **Results.** 805 patients were enrolled and entered Part 1. At study entry, most patients were male (63%) and Caucasian (75%); 16% were of Japanese/East Asian/Southeast Asian heritage. The median age was 52 years (range, 22-83). Most patients (79%) had advanced fibrosis or cirrhosis (FibroSURE® score equivalent to METAVIR F3/F4). The median duration of eltrombopag treatment during Part 1 was 21 days. Median platelets at baseline were 59,000/μL (range, 47,000-95,000/μL); these increased to a median of 93,000/μL (range, 9,000-489,000/μL) by week 2 of eltrombopag treatment and remained consistently elevated throughout Part 1 (Figure). Following a median of 2.8 weeks of treatment (range, 0.1-14.9 weeks), 773 patients (96%) achieved platelet counts ≥100,000/μL. Eltrombopag treatment was discontinued during Part 1 for 48 (6%) patients due to: platelets <100,000/μL (n=13); lost to follow-up (n=12); investigator discretion (n=10); adverse events (AEs, n=5); protocol deviation (n=5); or patient decision (n=3). During Part 2, 758 patients (94%) initiated antiviral treatment, the majority with eltrombopag doses of 25 mg/day (55%) or 50 mg/day (26%). The most common AEs observed during open-label treatment were headache (4%), diarrhea (3%), nausea (3%), and fatigue (2%). Two AEs (neither reported as related to eltrombopag treatment) resulted in fatal outcome (hepatorenal syndrome, malignant hepatic neoplasm). **Summary and Conclusions.** Eltrombopag treatment resulted in sustained platelet increases during the open-label, pre-antiviral treatment phase (Part 1) and was generally well-tolerated. Platelet increases were seen as early as 2 weeks following initiation of treatment. The vast majority of patients (96%) achieved platelet increases to ≥100,000/μL, the threshold for initiating PEG-2b plus ribavirin therapy, enabling a significant increase in SVR for eltrombopag- versus placebo-treated patients during Part 2 (Dusheiko 2011).

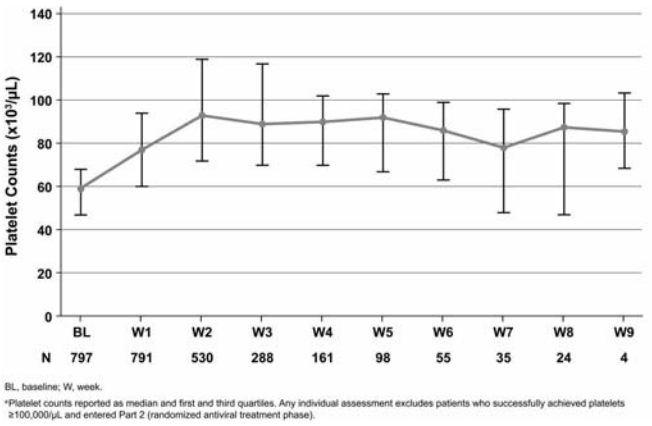

Figure 1. Platelet Counts During the Eltrombopag Open-Label Treatment Phase of ENABLE 2

0610

RITUXIMAB FOR THE TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA: THE UCLH EXPERIENCE

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Background. Rituximab therapy represents an important advance in the treatment of antibody-mediated Thrombotic Thrombocytopenic Purpura (TTP), and is effective in a variety of settings, including acute *de novo*, refractory and relapsing disease. More recently it has been used to treat electively patients at high risk of relapse, identified by a drop in ADAMTS13 activity and/or rise in IgG anti-ADAMTS13 antibody level. **Aims.** To describe and evaluate the use of rituximab in a tertiary referral unit for TTP: for patients treated for acute TTP, and elective rituximab to prevent TTP relapse. **Methods.** Review of patients referred for TTP treatment, given rituximab between 2004-2011, and determination of the timing and frequency of rituximab administration, response to treatment and rates of relapse.ResultsBetween 2004-2011, 95 patients received rituximab at our centre, as part of treatment for acute TTP; none had previously received rituximab. 74 received rituximab for acute *de novo* TTP (78%) and 21 for relapsed TTP (22%). Median age was 43 (range 12-81), female/male ratio was 2:1 (65F, 30M). 62 were Caucasian, 17 Afro-Caribbean and 16

Indian/Asian.61 patients received their first rituximab dose within 3 days of diagnosis of TTP, and 34 (with refractory TTP) received rituximab after 3 days. 71 received 4 infusions (375 mg/m^2), 15 received 6-8. The median number of Plasma Exchange (PEX) to remission was 17 (3.5-40) with a median time to sustained platelet recovery of 14 days (4-52). 5 patients died during acute admission. 13 of the remaining 90 patients relapsed (14%), with a median time to relapse of 24 months (3-49).In patients receiving rituximab within 3 days of admission/diagnosis (n=61), there was a significant reduction in number of PEX (16 vs. 21, p=0.037) and length of admission (15 vs. 21 days, p=0.011), compared to patients given rituximab after 3 days (n=34). There was a significant reduction in median time to platelet recovery (12.5 vs. 19.5 days, p=0.005) but no difference in time to relapse (22 vs. 24 months, p=0.435). Eight patients who had relapsed following previous treatment with rituximab received further rituximab: of these, 2 had a 3RD relapse (at 22 and 29 months), 1 died during relapse, and the remaining 5 remain in remission (median follow-up 28 months).14 patients, with previous acute TTP relapses and normal ADAMTS 13 in remission, received rituximab electively when ADAMTS13 activity dropped to <5%. Rituximab was given weekly at a dose of 375mg/m^2 for 4 doses in all but one patient, who received 100mg/m^2. All patients responded, with recovery of ADAMTS13 activity into the normal range in all but one case. Although 2 patients have subsequently required 2 further courses of rituximab each, none have had an acute TTP relapse. **Summary and Conclusions.** In our extensive cohort of patients with acute TTP, rituximab given within 3 days of admission is associated with reduced PEX procedures, shorter admission and a shorter time to platelet recovery. Relapse following Rituximab was 14%, median 2 years. Elective rituximab appears effective in preventing florid relapse in patients with very low ADAMTS13 levels.

0611

ROMIPLOSTIM FOR THE TREATMENT OF ADULTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ROUTINE CLINICAL PRACTICE - INTERIM RESULTS FROM A LARGE, EUROPEAN, OBSERVATIONAL STUDY

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Background. The thrombopoietin-receptor agonist romiplostim is recommended for second-line treatment of adult ITP, where the treatment goal is sustained increases in platelet counts to safe levels (Provan et al, 2010). Registrational studies of romiplostim in this setting were conducted in selected patient populations, and may not reflect routine clinical practice. **Aims.** Describe the use of romiplostim for the treatment of adult ITP in clinical practice. **Methods.** This ongoing, European, observational study enrolls ITP patients ≥18 years old, who have received romiplostim in clinical practice. Patients participating in another study, or who initiated romiplostim prior to commercial launch or have received other thrombopoietin-receptor agonists or related products are excluded. Data recorded as per clinical practice is collected for up to 2 years following romiplostim initiation, including any concomitant medications prescribed. Study outcomes include patient characteristics (at romiplostim initiation), romiplostim dose and adverse drug reactions (ADRs), summarized for patients meeting the study inclusion criteria (Full Analysis Set; FAS). We report data from an interim analysis conducted in September 2011. **Results.** 217 patients had enrolled, with 209 included in the FAS. Of these, 85% (178/209) remained on study, 9% (18/209) had completed the observation period and 6% (13/209) had withdrawn, with death the most common reason (11/209 [5%]). Median (Q1, Q3) age was 62.0 (47.0, 74.0) years, median weight 74.00 (64.00, 85.00) kg, and median baseline platelet count 18.0 (8.0, 33.0) x 10^9/L. One-third (70/209) of patients were splenectomised, 56% (116/209) female, and 76% (158/209) had received ≥3 prior ITP therapies. Median (Q1, Q3) time from ITP diagnosis was longer for splenectomised than non-splenectomised patients (9.75 [4.66, 22.18] *versus* 1.66 [0.22, 6.00] years). Median (Q1, Q3) duration of romiplostim exposure was 48.6 (20.1, 74.0) weeks (maximum 106 weeks), with romiplostim initiated at 1 and ≥3 µg/kg/week in 72% (150/209) and 16% (33/209) of patients. Taking the average weekly dose of all patients, the median (Q1, Q3) was 2.9 (1.5, 4.1) µg/kg/week. Platelet counts rose rapidly during the first 4 weeks of romiplostim treatment and remained >50 x 10^9/L thereafter (approximately 2 years). The most commonly reported ADRs were headache, thrombocytosis, arthralgia, asthenia, flushing and myalgia (2.6-7.3 events per 100 subject-years of exposure). Seven serious ADRs were reported: myelofibrosis (2 events, where the initial disease diagnosis was inconsistent with ITP and myelofibrosis more likely due to the underlying disease [MDS, metastases to bone marrow]); pulmonary embolism (2 events); drug ineffective (1 event); platelet count decreased (1 event, platelets <20 x 10^9/L); (reversible) thrombocytosis (1 event, platelets 477 x 10^9/L). No fatal ADRs were reported. **Summary and Conclusions.** At the time of this analysis, patients receiving romiplostim in clinical practice tended to be older and more heavily pre-treated than those enrolled in previous registrational studies. With similar doses as previously reported (Kuter et al, 2008), and no new safety signals, splenectomised and non-splenectomised patients with ITP of varying duration achieved sustained increases in platelet counts to safe levels. With the implementation of treatment guidelines, clinical practice will likely evolve to include younger, less heavily pre-treated patients.

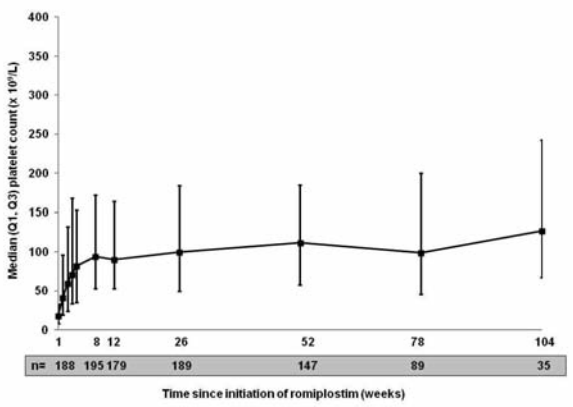

Figure 1. Platelet counts over time.

0612

STANDARDIZATION OF BLEEDING ASSESSMENT IN IMMUNE THROMBOCYTOPENIA (ITP): A PRELIMINARY REPORT FROM THE INTERNATIONAL WORKING GROUP ON ITP (IWG ON ITP)

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Background. In its report on standardization of terminology, definitions and outcome criteria in ITP (Rodeghiero et al, Blood 2009), the IWG deferred the def-

inition of the bleeding phenotype and the grading of its severity to a subsequent report. The IWG acknowledged that treatment response should ideally reflect clinical endpoints, including bleeding and quality of life, rather than rely exclusively on surrogate endpoints (platelet count) with arbitrary thresholds. **Aims.** To provide a consensus-based bleeding assessment tool inclusive of standardized definitions of bleeding, a symptom-specific and domain-specific grading and a harmonized standardized questionnaire for adults and children. **Methods.** The main issues were agreed-on and a final consensus was reached after several rounds of Delphi-like questionnaire and three face-to-face meetings. **Results.** *1. Definitions and terminology.* Definitions are consistent with those adopted for other bleeding disorders and are relevant for the purpose of developing an ITP-specific bleeding assessment. *2. Classification of bleeding symptoms*. Bleeding signs/symptoms are grouped according to three major domains: Skin (S), visible Mucosae (M) and Organs (O). Skin (epidermis, dermis and subcutaneous tissues) includes Petechiae, Ecchymosis (purpuric macula, bruise or contusion), Subcutaneous hematomas, Bleeding from minor wounds. Visible Mucosae include Epistaxis, Oral cavity (gum bleeding, hemorrhagic bullae/blisters, bleeding from bites to lips & tongue or after deciduous teeth loss/extraction), Subconjunctival hemorrhage. Organs include GI bleeding (hematemesis, melena, hematochezia, rectorrhagia), Lung bleeding (hemoptysis), Hematuria, Menorrhagia, Muscle hematoma, Hemarthrosis, Ocular bleeding, Intracranial (intracerebral, intraventricular, subarachnoidal, subdural, extradural). 3. *Gradation of bleeding severity (SMOG system)*. Each bleeding manifestation should be assessed at the time of examination. Its severity is graded from 0 to 4 (see example in table). Appreciation of bleeding based on history only, without supporting medical documentation, will be given a grade 1 designation. Within each domain, the same grading is assigned to the symptoms judged to have similar clinical relevance. For each symptom, the worst ever episode during the observation period is graded and then the worst episode within the domain is recorded. For example if the highest grade is 2 for skin, 3 for mucosae and 2 for organs, SMOG is S2M3O2. The index produced by summing the worst ever grade in the 3 domains represents the final score for that particular patient. In the example shown, the final score is 7. However, it is immediately appreciated that O2 has a much higher clinical impact than S2, so that a different weight (to be determined by ad hoc prospective studies) should be attributed to the different domains. *4. Questionnaire,* harmonized to the different bleeding manifestations and their grading, to facilitate the allocation of each symptom and its severity. **Conclusions.** The strength of this SMOG system lies in the agreement of a worldwide group of experts and should fill the existing gap in having a reliable and clinically relevant description of the bleeding phenotype of patients with ITP and its modifications by the various available treatments. The use of this tool for the purpose of decision making is discouraged until its value has been validated by appropriately designed prospective studies.

Type of bleeding	GRADES BASED ON THE WORST EVER EPISODE DURING THE OBSERVATION PERIOD				
	0	1	2	3	4
MUCOSAL					
Epistaxis	□ No/ unchanged/lasting < 5 min	□ Lasting > 5 min. or interfering with daily activities □ Any episode if based on patient history only	□ Antifibrinolytics or procedures by a physician not requiring packing or cauterization	□ Packing or cauterization or requiring in-hospital evaluation	□ RBC transf.
Oral cavity – gum bleeding	□ No/ unchanged/lasting < 10 min	□ Lasting > 10 min. or interfering with daily activities □ Any episode if based on patient history only	□ Antifibrinolytics or procedures by a physician not requiring hospitalization	□ Requiring in-hospital evaluation	□ RBC transf.

Figure 1.

POSTER SESSION II

Acute lymphoblastic leukemia - Biology 2

0613

SET-UP OF A MULTIPLEX PCR TO RAPIDLY DETECT IKZF1 (IKAROS) GENE BREAKPOINT DELETION IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background. During the last five years, *IKZF1* has been established as one of the most clinically relevant tumor suppressors in ALL. Deletion of a single *IKZF1* allele or mutations of a single copy of IKZF1 were firstly detected in 15% of all cases of pediatric B-cell ALL and in more than 80% of Ph+ lymphoid leukemia cases, either *de novo* Ph+ ALL or chronic myeloid leukemia at progression to lymphoid blast crisis. The deletions either involve the entire *IKZF1* locus, resulting in loss of function, or delete an internal subset of exons, resulting in the expression of dominant negative isoforms. The detection of *IKZF1* alterations at diagnosis might be useful in identifying patients with a high risk of treatment failure. *To better* stratify ALL patients according to *IKZF1* status, we set-up, validated and assessed the routine applicability of a *IKZF1* deletion screening strategy based on a multiplex-PCR. **Patients and Methods.** We studied 136 adult BCR-ABL1-positive and negative ALL patients. For each type of common *IKZF1* deletion (Δ4-7, Δ2-7, Δ4-8) an appropriate pair of primers will be designed using Primer 3 (http://frodo.wi.mit.edu/primer3/). Their ability to efficiently amplify the deletion was tested. PCR amplifications was performed using 50 ng genomic DNA as template for each reaction and a FastStart Taq DNA Polymerase (Roche Diagnostics). For each patient, 3 amplicons were generated and sequenced (amplicon A for Δ4-7, amplicon B for Δ2-7 and amplicon C for Δ4-8). Moreover, a multiplex amplification strategy was assessed and a forth amplicon was generated and sequenced (forward primers were localized on intron 1 and 3, the reverse ones in the intron 7 and at the end of the gene, after the exon 8). The size of amplicons depends on the positions of the breakpoints in the *IKZF1* gene and on the number of nucleotides added at the conjunction. It was in the range of 450-600 nucleotides. Positive controls were used for each run. **Results.** On 136 patients previously analyzed by SNPs array, we identified *IKZF1* deletions in 108/136 (79.4%) samples. Deletions were: 54 Δ4-7, 31 Δ2-7 and 4 Δ4-8; in 16 cases the deletion was extended to all gene, and 10 patients presented two breakpoints simultaneously. Multiplex PCR and subsequent sequencing were performed for most common deletions in 40 patients confirming previous results and indentifying the precise genomic positions of breakpoints and the nucleotides added at the conjunction. This rearrangement has been used to strictly monitor Minimal Residual Disease (MDR) during the follow-up and at relapse to confirm the clonal fidelity. **Conclusions.** We set-up a rapid and sensitive method using less amount of DNA sample, to screen ALL patients at diagnosis and to monitor MRD during the treatment. **Supported by**: European LeukemiaNet, AIL, AIRC, FIRB 2006, PRIN 2008, Fondazione del Monte di Bologna e Ravenna, Strategico di Ateneo, GIMEMA Onlus.

0614

INTERNATIONAL STANDARDIZATION OF MINIMAL RESIDUAL DISEASE ASSESSMENT FOR PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA EXPRESSING MINOR-BCR-ABL: UPDATED RESULTS OF EUROMRD

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Background. Minimal residual disease is well established as an important prognostic parameter and is used for stratification and treatment decisions in acute lymphoblastic leukaemia (ALL). DNA- based PCR analysis of Ig-/TCR-rearrangements have been well standardised during the last decade, resulting in robust systems for MRD analysis with an intra- and inter-assay variability of less than half a log. In Ph+ALL, MRD analysis relies on RNA-based RT-PCR techniques that are highly sensitive but as yet lack standardisation between laboratories. Since laboratories differ substantially in both their methodology and analysis strategy, interpretation and meaningful comparison of results between laboratories and from different studies is difficult or impossible. Moreover, and in contrast to CML, there are no generally accepted definitions of molecular response that could identify thresholds on which to base therapeutic decisions. **Aims.** To assess *i)* the variability of BCR-ABL quantification between laboratories with recognized expertise in bcr-abl analysis, *ii)* identify optimal laboratory methods and iii) to standardize analysis and interpretation of RT-PCR results for Ph+ALL, the EWALL and ESG-MRD-ALL consortia initiated a multinational project for quality assurance involving more than 30 laboratories from 14 countries worldwide. We here report the results of the first seven m-BCR-ABL laboratory control rounds, performed between march 2008 and march 2012, that focussed on inter-intraassay variability of rt-PR techniques, comparison of housekeeping genes, plasmids, platforms and reagents. **Methods.** Serial dilutions of the BCR-ABL positive cell line Sup B15 with the BCR-ABL negative cell line Nalm 6 covering 5 logs were produced. In the 7 lab rounds, 1355 aliquots, each generated with a total amount of 5 x 10E+06 cells in a final volume of 1 ml were produced, stabilized in TRIZOL or buffer RLT and frozen at -20°C until shipment or centralized isolation of RNA and reverse transcribed. Participants were asked to process the material using predefined procedures. **Results.** In the first QC round a major finding was the high variability in RNA-yield between laboratories despite using the same extraction method, with an up to 3 log difference in ABL copy numbers. In addition, there was rare false-positivity and false negativity (specifity 94.8%). With centrally produced cDNA the variability improved and there was a lower frequency of false-positivity reaching 100% specifity in QC 2. By using standardized assays, in 12 of 33 laboratories no conversion factor is needed, 20 of 33 laboratories are at the moment within one log difference when comparing the BCR-ABL/ABL ratios. Comparison of housekeeping genes (ABL/GUS) revealed non-linearity at high transcript levels resulting in an underestimation of intraindividual log reductions and may be relevant when assessing disease prognosis. The quantitative range was generally above 10E-04. **Conclusions.** Initiation of laboratory control rounds and implementation of standardized methodology improved the variability and reduced the frequency of false-positive results. Sensitivity of current techniques is still unsatisfactory for diseases with aggressive growth kinetics such as Ph*ALL. Further standardisation of technical and analytical methodology

0615

FREQUENCY AND CLINICAL PRESENTATION OF EZH2 MUTATIONS IN CHILDHOOD ACUTE LEUKEMIA

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Background. There is growing evidence that epigenetic deregulation plays an important role in the pathogenesis of both childhood and adult leukemia. Somatic mutations of the histone methyltransferase gene *EZH2* have recently been described in myeloid and lymphoid malignancies in adults and seem to be associated with poor prognosis. EZH2 catalyses trimethylation of histone 3 lysine 27 (H3K27) resulting in transcriptional repression of genes involved in development, stem cell maintenance and differentiation. **Aims.** We sought to determine the frequency and prognostic impact of *EZH2* mutations in a clinical well-defined cohort of 200 randomly selected childhood acute leukemia patients: acute myeloid leukemia (AML), n=75; B-lineage acute lymphoblastic leukemia (B-lineage ALL), n= 85; T-cell acute lymphoblastic leukemia (T-ALL), n=40. **Methods.** Genomic DNA was isolated from bone marrow cells at diagnosis. PCRs were performed using standard conditions with primers covering all 20 exons of the *EZH2* gene, including its intron-exon boundaries to detect potential splice mutations. Mutation analysis was performed by Sanger sequencing. **Results.** All 75 childhood AML and 48 B-lineage ALL cases have been analyzed so far. *EZH2* mutation status of the entire cohort will be presented at the meeting. To date, we identified two *EZH2* mutations, one each in AML and B-lineage ALL children. The AML patient was a 16-year-old girl (FAB type M2) without Auer rods and central nervous system involvement but confirmation of leukemic blasts in bilateral pleural effusions. Cytogenetic analysis showed the karyotype 45,X,t(8;21)(q22;q22). The patient was treated within the high-risk group of the German AML-BFM 93 trial, showed continuous complete remission but suffered from repetitive *Aspergillus fumigatus* infections and died due to a severe *Aspergillus* sepsis. The mutation is a homozygous in-frame insertion of 6 bp within *EZH2* exon 20. On protein level, the mutation inserts the two amino acids lysine and threonine at position 743 and 744 (SET domain), respectively, resulting in an abnormal EZH2 protein with a length of 753 amino acids. Analysis of a subsequent remission sample showed an absence of the mutation indicating that the *EZH2* mutation is somatically acquired. The patient with B-lineage ALL was a 10-year-old girl classified as a pro-B-ALL by immunophenotyping and treated within the German ALL-VII/81 trial. She initially achieved complete remission but relapsed and died 6 months after start of treatment. The underlying mutation is a heterozygous frameshift deletion of 3 bp within *EZH2* exon 6 that causes a change of the protein sequence starting from amino acid D184 and thus affects function of three major EZH2 domains (D2, CXC, SET). **Summary and Conclusions.** To our knowledge, this is the first report to describe *EZH2* mutations in pediatric acute leukemia patients. Although *EZH2* mutations seem to be rare in childhood leukemia our findings indicate that *EZH2* mutations might contribute to the disease in specific cases. The incidence of *EZH2* mutations in childhood acute leukemia needs to be assessed in a larger cohort of patients.

0616

DISTINCT GENETICS OF TEENAGE AND YOUNG ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background. The outcome for Teenagers and Young Adults (TYA) with T-cell Acute Lymphoblastic Leukaemia (T-ALL) has improved significantly using paediatric based chemotherapy protocols, although it remains inferior to the outcome for children aged 1-9 years at diagnosis. This difference in outcome remains poorly understood. The genetic make-up of T-ALL is currently under investigation to establish the full repertoire of oncogenic events required to develop this aggressive disease. Comparison of genetic drivers identified in Teenagers and Young Adults with those in younger children would establish whether age related differences in leukaemia genetics could in part explain the observed difference in outcome. This genetic characterisation will at the same time identify drivers in those patients with an apparently normal karyotype. Current risk stratification in T-ALL is based on age and white cell count at presentation only. Thus these molecular studies have the potential to identify high risk patients to improve risk stratification. **Aims.** (1) To genetically characterise TYA T-ALL and establish whether T-ALL in Teenagers and Young Adults is a distinct disease entity; (2) Catalogue the comprehensive repertoire of collaborating genetic events required to lead to TYA T-ALL; (3) Correlate the genetic abnormalities with outcome. **Methods.** We accessed viable cells stored at diagnosis of TYA T-ALL (10-25 years) and extracted RNA and DNA. The patient DNA was examined at high resolution for Copy Number Alterations (CNAs) and Loss of Heterozygosity (LOH) using the Affymetrix SNP6.0 platform. The array data was analysed using CNAG 3.3.0.1 and Partek GS 6.6. The most frequent CNAs were confirmed by MLPA (P-383-T-ALL). In addition the DNA was screened for mutations in *NRAS, KRAS, CBL, FLT3* and *SHP2* using denaturing high performance liquid chromatography (dHPLC) on a Transgenomic WAVE machine using (whole genome amplified) genomic DNA. The RNA was transcribed to cDNA and will be subjected to real-time PCR for *CDKN2A* gene expression. Promoter methylation was performed by SABiosciences EpiTect Methyl qPCR Arrays and Methylation Specific PCR (MSP). **Results.** The most frequent CNA identified was deletion of *CDKN2A* in 72.7% of patient samples (91.6% homozygous deletions). Other frequent CNAs were loss of *MLLT3* (31%), *STIL* (27%), *PTEN* (19%), *LEF1* (15%) and gain of *MYB* (12%). These CNAs were confirmed by MLPA analysis. The only recurrent region of Copy Number Neutral LOH encompassed 9p24.3-p13.3, including the gene *CDKN2A* (27%). 2 Mutations have been identified; 1 *NRAS* and 1 *CBL* mutation (known variants). We demonstrated *CDKN2A* promoter methylation in cases without gene deletion by Methylation Specific PCR, but not Epitect Methyl Arrays, which indicates *CDKN2A* gene alteration in the majority of patient samples.Summary: Genomic analysis to date has not shown significant differences between T-ALL in children versus Teenagers and Young Adults. We are in the process of analysing additional cases for CNAs, LOH, including *NOTCH1/FBXW7/PTEN* gene mutations. To be able to appreciate potential cooperation between genetic abnormalities, we are complementing the DNA analysis with the measurement of gene expression of the oncogenes *TAL1, LYL1, LMO1, LMO2, TLX1, TLX3, NKX2, ERG* and *MEF2C*.

0617

CAMP-INDUCED P53 DEGRADATION IS INDEPENDENT OF EXCHANGE PROTEIN ACTIVATED BY CAMP IN PRE-B ALL CELLS

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cAMP-induced p53 degradation is independent of exchange protein activated by cAMP in pre-B ALL cells. **Background.** The p53 tumor suppressor protein is a potent roadblock to tumor development. Cells that are insulted by chemotherapeutic DNA-damaging agents or other forms of stress stabilize the p53 protein by phosphorylation or other modifications. Stabilized p53 accumulates in the nucleus to regulate the expression of numerous pro-apoptotic genes. **Aims.** The aim of this study was to investigate the inhibitory role of cyclic adenosine monophosphate (cAMP) levels on p53 protein in acute lymphoblastic leukemia (ALL) cells. More importantly, we were interested to show through which receptor cAMP acts to promote p53 degradation. **Methods.** In cell cultures, we investigated the effects of cAMP-increasing agents forskolin/IBMX on stimulated p53 of ALL cell lines. Western blotting analysis was performed to detect phosphorylation and acetylation state of p53 protein, total p53, phospho-cAMP response element-binding protein (CREB), and the levels of other proteins which were involved in doxorubicin-induced apoptosis. Flow cytometry was applied to analyze apoptosis. The gene expression of p53 and its target genes was examined by real-time polymerase chain reaction. **Results.** These results indicate that elevation of cAMP levels in ALL cells exposed to DNA damage attenuates p53 accumulation. We further demonstrate that elevated cAMP levels repressed DNA damage-induced p53 protein phosphorylation and acetylation in NALM-6 cells. Increased cAMP levels also shifted the ratio of the death promoter to death repressor genes via alteration of Bcl-2 and Bax proteins expression. Inhibition of proteosome function with MG-132 reversed the inhibitory effect of cAMP on p53. However, targeting the p53-Mdm2 interaction did not rescue accumulated p53 from the destabilizing signal of cAMP. The specific agonist of the cAMP receptor exchange protein activated by cAMP had no effect on p53 expression in doxorubicin-treated NALM-6 cells, whereas PKA activators decreased p53 accumulation. **Summary and Conclusions.** In conclusion, our studies demonstrate that cAMP-PKA pathway regulates the sensitivity toward DNA-damaging agents via inhibition of a p53-dependent pathway in B-cell precursor ALL (BCP-ALL) cells.

0618

PHENOTYPE AND TARGET EXPRESSION PROFILES OF LEUKEMIC STEM CELLS IN PH+ AND PH- ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Acute lymphoblastic leukemia (ALL) is a life-threatening hematopoietic neoplasm characterized by abnormal growth and accumulation of lymphoblasts in various hematopoietic tissues. In a substantial number of patients, the Philadelphia (Ph) chromosome and the related oncoprotein BCR/ABL, are detectable. Despite recent improvements in therapy and use of BCR/ABL tyrosine kinase inhibitors (TKI), the prognosis is still poor. During the past few years, several attempts have been made to improve targeted treatment approaches in ALL. One strategy is to define markers and targets expressed on leukemic stem cells (LSC) in these patients. At least in Ph+ ALL, the leukemia-initiating cells are considered to reside within a CD34+/CD38- fraction of the clone. In the present study, we examined the expression of various stem cell markers and target antigens in CD34+/CD38-/CD123+ stem cells in patients with Ph+ ALL (n=4), Ph- ALL (n=8), Ph+ CML (n=20), and in control bone marrow (BM) samples (unexplained cytopenia, n=10). Surface expression of target antigens was analyzed by multicolor flow cytometry, and mRNA expression levels by qPCR. As assessed by flow cytometry, CD34+/CD38-/CD123+ cells were found to co-express CD19, CD44, CD52, CD133, CD135, and CXCR4 in all ALL patients examined. In most ALL patients tested (9/12), LSC also expressed CD33. In CML, LSC were found to express a similar profile of antigens, including CD33, CD44, CD52, CD133, CD135, and CXCR4, but not CD19. In control BM samples, CD34+/CD38- cells expressed the same antigens, however, the levels of CD33 and CD52 were lower compared to LSC in ALL and CML. The IL-1RAP was found to be expressed on LSC in Ph+ CML and in Ph+ ALL, but not on LSC in Ph- ALL or in normal BM stem cells. By contrast, the SCF receptor KIT (CD117) was expressed on LSC in Ph+ CML and in Ph- ALL, but was hardly detectable on LSC in Ph+ ALL. The IL-2RA (CD25) and the SDF-1-degrading surface enzyme dipeptidyl-peptidase IV (DPPIV=CD26) were found to be expressed on LSC in all patients with Ph+ CML and in a subset of patients with Ph+ ALL, namely those in whom BCR/ABL-p210 was detectable, whereas in patients with Ph+ ALL with BCR/ABL-p190 as well in Ph- ALL or normal BM samples, CD34+/CD38- cells did not co-express CD25 or CD26. The target receptor CD20 was not detectable on LSC in any of the patients´ cohorts analyzed, including Ph+ and Ph- ALL. In most instances, surface expression of target antigens could be confirmed by qPCR. Together, our data show that LSC in Ph+ ALL and Ph- ALL express a unique phenotype, including major surface targets. In Ph+ ALL with BCR/ABL-p210, the phenotype of ALL LSC closely resembles the phenotype of LSC in Ph+ CML, confirming the close relationship and similar pathogenesis of these two leukemic conditions.

0619

TRANSMEMBRANE ADAPTOR PROTEIN NTAL ENHANCES PROXIMAL SIGNALING AND POTENTIATES CORTICOSTEROID INDUCED APOPTOSIS IN T-ALL

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Background. The biology of T-lineage acute lymphoblastic leukaemia (T-ALL) is characterized by the pre T-cell receptor (TCR) signaling. Transmembrane adaptor proteins could play an important role in the development of T- ALL. LAT is the adaptor protein essential for signal transmission from TCR. NTAL is an analogue of LAT except for a binding site for PCKgamma. **Aims.** We analysed the impact of NTAL and LAT expression on biology and treatment response in T-ALL. **Methods**. NTAL and LAT expression was analysed in diagnostic bone marrow samples of 39 pediatric patients with T-ALL using qRT-PCR and flow cytometry. Jurkat cell line (T-ALL cell line expressing no NTAL) (Jurkat/wt) and derived Jurkat cell line with stable NTAL expression (Jurkat/NTAL+) were used for in-vitro experiments. Cell signalling and cell death after TCR stimulation and after methylprednisolone treatment were analysed using flow cytometry, Western blot and qRT-PCR. **Results.** Leukemic blasts of T-ALL patients responding favourably to initial prednisone treatment had higher levels of NTAL than patients responding unfavourably (p=0.028) whereas LAT expression was stable (p>0.9). This observation was confirmed in in-vitro experiment - after 48 hours of methylprednisolone treatment the percentage of surviving Jurkat/NTAL+ vs. Jurkat/wt cells was 11% vs. 31% (p<0.05). Moreover, Jurkat/NTAL+ cells were more sensitive to TCR induced cell death than Jurkat/wt cells (40.7% vs. 61.0% surviving cells at 24 hours after TCR stimulation, p<0.05). Jurkat/NTAL+ cells showed significantly higher levels of ERK phosphorylation after TCR stimulation (median 1.5 fold, p<0.05) and of CD69 activation marker (90.5% vs. 77.4%, p<0.05) compared to Jurkat/wt, whereas phosphorylation of JNK showed an inverse pattern and phosphorylation of p38 MAPK was stable. The ERK inhibitor U0126 almost completely abrogated TCR induced cell death and reversed sensitizing effect of the NTAL protein to methylprednisolone induced cell death - percentage of surviving Jurkat/NTAL+ and Jurkat/wt cells after 48 hours of coincubation with methylprednisolone and U0126 is 29.3% and 29.9% respectively (p=0.49). **Conslusions**. We conclude that in our experiment NTAL is a tumor suppressor enhancing ERK phosphorylation of leukemic blasts. ERK molecule is the key protein responsible for increasing cell sensitivity to methylprednisolone induced cell death.This study was supported by grants NS/10473-3, MSM 0021620813 and MSMT NPV 2B06064.

0620

CHANGES OF HEMATOPOIETIC MICROENVIRNMENT AND LYMPHOCYTES IN HEMATOPOIETIC STEM CELL TRANSPLANTATION OF ACUTE LYMPHOBLASTIC LEUKEMIA: COMPARISION BETWEEN RELAPSE AND NON-RELAPSE GROUPS

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Background. Hematopoietic microenvirnment is essential for the recovery of the hematopoiesis after chemotherapy or hematopoietic stem cell (HSC) transplantation (HSCT). However, the exact interaction between HSCs and their microenvironment is not fully understood. The reconstitution of lymphocytes after chemotherapy or HSCT is also associated with hemopoietic engraftment. The delayed reconstitution of lymphocytes is related with relapse of malignancy. We analyzed markers of HSCs, hematopoietic microenvirnment and lymphocytes at regular intervals and evaluated the changes of them before and after HSCT to know the differences between engraftment and relapse groups. **Methods.** The immunohistochemistry for HSCs (CD34 and CD117), HSC microenvironment (osteonectin, CXCL12 and CXCR4), and lymphocytes (CD3, CD4, CD8, CD20 and CD56) was performed on bone marrow (BM) biopsies or clot sections, from the control group (8 lymphoma patients without BM involvement), non-relapse group (8 ALL patients with engraftment after HSCT) and relapse group (9 ALL patients with relapse after HSCT), at initial diagnosis (Dx), 1st complete remission (CR) after induction chemotherapy, before HSCT (pre-HSCT), and 1-3 months (after 1-3m) or 6-12 months after HSCT (after 6-12m). The positive cells for each marker were counted on 10 high power fields (x400) by microscopy, and then the average number of 10 fields was corrected with BM cellularity. **Results.** CD34 at Dx was more highly expressed in the patient groups (p<0.001), and CD117, osteonectin and CXCL12 expression was lower in the patient groups than in the control group (p=0.016, p<0.001, and p<0.001, respectively). Osteonectin from Dx to pre-HSCT in both non-relapse and relapse group were lower than in control group. At CR, pre-HSCT, and HSCT 1-3, CXCL12 expression was higher in the non-relapse group than in the relapse group (p=0.015, p=0.046, and p=0.015, respectively). With the exception of $CD56^+$ NK cells, the numbers of all lymphocytes (CD3+, CD4+, CD8+, and CD20+ cells) decreased in patient groups at Dx (p<0.001, p<0.001, p=0.001, and p=0.002, respectively). CD3, CD4, and CD8 expression were significantly higher in the non-relapse group than in the relapse group at pre-HSCT (p<0.001, p=0.001, and p<0.001, respectively). CD3 showed significantly higher expression in the non-relapse group at HSCT 1-3 and HSCT 6-12 (p=0.006 and p=0.033, respectively). At HSCT 6-12, CD4 expression also increased in the non-relapse group (p=0.017). **Conclusions.** We confirmed that BM T cells and T cell subsets in patients with ALL were reduced in the both periods of pre- and post-HSCT, and T lymphocytes in relapse group were less than those in non-relapse group. It was suggested that T lymphocytes played major roles to prevent from relapsing ALL, however, roles of NK cells in BM seemed to be insignificant. We thought that CD34 in ALL might be helpful in predicting the relapse of ALL following HSCT, and osteonectin might play a role as tumor suppressor in ALL. We also confirmed that CXCL12 had an important role to regenerate BM and a reduction of it might be a predictor as a relapse of ALL.

0621

SHRNA-MEDIATED BAALC KNOCKDOWN AFFECTS PROLIFERATION AND APOPTOSIS IN HUMAN ACUTE MYELOID LEUKEMIA CELLS

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Background. Brain and acute leukemia, cytoplasmic (BAALC) gene, encodes a protein with no homology to known proteins or functional domains, is a novel molecular marker indicating the inferior outcome in acute myeloid leukemia (AML) with normal cytogenetics. But the biological function of BAALC is largely unknown. The functional studies investigating the impact of BAALC-expression in leukemia cell lines could not demonstrate a mechanistic role of BAALC on proliferation and apoptosis. **Aims.** To investigate the effect of small hair RNA (shRNA) -mediated BAALC gene knockdown on proliferation and apoptosis in human acute myeloid leukemia cells KG1a. **Methods.** The BAALC shRNA-expressing plasmid was constructed using the pGPU6/GFP/Neo vector, shRNA-NC and shRNA-GAPDH plasmid were also constructed to act as a negative and positive control separately. The shRNA expressing plasmid was transfected into KG1a cells using Nucleofector system. Total RNA and protein of transfected KG1a cells were extracted to verify the silence efficacy by RT-PCR and western blot. Silence of BAALC on proliferation of KG1a cells was detected by CCK-8 assays; the apoptotic nuclear changes after stained with DAPI was visualized by fluorescence microscopy and the population of apoptotic cells was determined by flow cytometric analysis. **Results.** RT-PCR showed that BAALC mRNA expression was markedly decreased in the pGPU6/GFP/Neo-BAALC-shRNA transfected KG1a cells. Consistently, the GAPDH mRNA levels in KG1a cells transfected with the positive control plasmid of shRNA-GAPDH were also decreased, whereas no change was observed in the pGPU6/GFP/Neo-shRNA-NC mock transfected cells. In line with the mRNA expression changes, the levels of BAALC protein were also remarkably inhibited in different pools of cells transfected with BAALC shRNA-expressing plasmid compared with those transfected with shRNA-NC-expressing plasmid. This suggested the BAALC gene expression in KG1a cells was stably silenced. Furthermore we investigated the impact of BAALC silence on proliferation and apoptosis. The growth curves of CCK8 assay showed proliferation of KG1a-BAALC shRNA cells was suppressed at different time point (48h, 72h, and 96h) when compared with the control groups. The apoptotic cellular nuclear changes (i.e., punctuated or granular and bright nuclei) were observed predominantly in the BAALC-shRNAs transfected but not shRNA-NC transfected cells. Annexin-V/PI flow cytometric analysis results showed that the apoptotic population of cells tranfected with BAALC-shRNA was significantly higher than those transfected with shRNA-NC (85.33% vs 12.67%, P<0.05). **Conclusions.** Our results showed that shRNA-mediated silence of BAALC inhibited proliferation and induced apoptosis of KG1a cells. Targeting BAALC may be a novel and effective approach for leukemia treatment. BAALC may act as an inferior prognostic factor through prompting proliferation and inhibiting apoptosis in leukemia cells.

0622

FORCED RIZ1 EXPRESSION INDUCED TUMOR GROWTH SUPPRESSION, CELL CYCLE ARREST AND INCREASE IN GATA3 EXPRESSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA OF T CELL PHENOTYPE

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Background. Retinoblastoma protein-interacting zinc finger gene (*RIZ1)* has characteristics of a negative regulator of tumorigenesis. We recently reported that *RIZ1* expression is decreased in adult acute lymphoblastic leukemia (ALL). Moreover, *RIZ1* methylation of the promoter was more frequent and decrease in *RIZ1* expression was more significant in T-ALL than in B-ALL. RIZ1 was isolated as a GATA3-binding protein G3B. GATA3 is a transcription factor known to be expressed very specifically in the T-cell lineage. **Aims.** (1) To elucidate the relevance of *GATA3* in adult ALL. (2) To examine the association between *RIZ1* and *GATA3* expressions in T-ALL cells. (3) To investigate whether forced *RIZ1* expression in T-ALL cell lines induce growth suppression and affect *GATA3* expression. **Methods.** We examined the *GATA3* expression by quantitative real-time reverse transcription-polymerase chain reaction (PCR) analysis in 70 newly diagnosed adult ALL patients (60 B-ALL and 10 T-ALL). Normal T lymphocytes (n = 3) and leukemic cell lines (n = 9) were also examined. Patient characteristics (age, sex, WBC count, LDH at diagnosis and karyotype) were investigated. Correlations between *RIZ1* expression and *GATA3* or clinical characteristics were analyzed with t tests. The human RIZ1 protein expression vector p3RIZRH1 was transfected into T-ALL cell lines (MOLT-4 and Jurkat) with the Nucleofector system (Amaxa, Gaithersburg, MD, USA). The transfected cells were cultured for 96 hours, and cell growth, cell cycle, apoptosis and *GATA3* expressions were analyzed. **Results.** The mean *GATA3* expression in T-ALL patients was 4.84 (n = 10) and 1.27 in B-ALL patients (n = 60). Similar to *RIZ1* in T-ALL, the mean *GATA3* expression in T-ALL patients (mean 4.84) was lower than that in T lymphocytes from healthy individuals (mean 19.28). In addition, the association between *RIZ1* (mean 0.61) and *GATA3* (mean 4.84) expressions in T-ALL patients was statistically significant ($P = 0.004$). We found no statistical differences between the *GATA3* expression and other clinical characteristics. *GATA3* expressions in 6 T-ALL cell lines (mean 1.40) were also decreased, compared with those of normal T lymphocytes (mean 19.28). The *GATA3* was not expressed in B lymphoid (Raji) or myeloid (K562 and KG1) cell lines. Forced *RIZ1* expression in T-ALL cell lines resulted in inhibition of cell growth, G2/M arrest and increase of apoptotic cells. *GATA3* expressions in these cells were increased in accordance with *RIZ1* re-expression in a time-dependent manner. **Summary and Conclusions.** Decreased expressions of the *RIZ1* gene are frequent in T-ALL. Growth suppression accompanied with G2/M arrest and apoptosis were found by *RIZ1* re-expression in T-ALL cell lines. These results suggest that *RIZ1* inactivation is involved in T-ALL pathogenesis. Moreover, as *GATA3* expression of all T-ALL patients was decreased and it was increased by *RIZ1* re-expression in the cell lines, decreased *RIZ1* expression may be associated with *GATA3* suppression. Although leukemogenesis of T-ALL is heterogeneous and it originated from various T lineage cells, *RIZ1* might be involved in the development of T-ALL through *GATA3* repression.

0623

GENETIC POLYMORPHISMS IN DNA REPAIR GENES AS MODULATORS OF ACUTE LEUKEMIA RISK

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Background Although the pathogenesis of Acute leukemia (AL) remains unknown, the results of epidemiologic studies suggest that heritable factors are important in terms of susceptibility. Polymorphisms in DNA repair genes may modify the individual's risk for genomic damage, and, as a consequence, the risk of developing malignant diseases. **Aims.** To investigate the relavent of single nucleotide polymorphism in DNA repair gene(XPA XPC XPD XRCC1 and Rad51)and genetic susceptility of acute leukemia. **Methods.** The authors evaluated the relation between polymorphisms in 3 nucleotide excisionrepair pathway genes (XPA [A23G], XPC [Ala499Val and Lys939Gln], and XPD [Lys751Gln]), the base excision repair XRCC1 (Arg194Trp and Arg399Gln), and homologous recombination repair Rad51 (G135C) in a chinese population of 554ALcases and 651 matched controls using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry . Variants were investigated independently and in combination; odd ratios (OR) and 95% confidence intervals (95% CIs) were calculated by logistic regression analysis and adjusted for the effect of sex and age. **Results.** There were no association between variation in the XPC Lys939Gln, XPD Lys751Gln, and XRCC1 Arg194Trp, Arg399Gln polymorphisms and acute leukemia. However there were significantly higher prevalence of the polymorphic variants RAD51G135C gene in acute promyelocytic leukemia (APL) cases and XPA-A23G gene in acute lymphoblastic leukemia (ALL) cases, when compared with controls, increasing the risk of APL 1.47-folds (95% CI: 1.04-2.07) and ALL 1.73-fold (95% CI: 1.01-2.97), respectively. The combined analysis demonstrated that Rad51/XPC and Rad51/XPD polymorphisms were associated with a significant increase in APL risk. The Rad51 135GC/CC and XPC 499CT/TT, or Rad51 135GC/CC and XPD 751GT/GG variant genotypes had ORs of 1.86 (95% CI, 1.2□'3.02) and 3.26 (95% CI, 1.49□'7.13), respectively. Similarly, Carriers of both the XPA-A23G and XPC-C499T variants were at highest ALL risk (OR:2.93; 95% CI: 1.11- 7.73). **Conclusions.** These findings indicate that XPA A23G and Rad51 G135C polymorphisms may be risk factors for ALL and APL in the Chinese population, respectively, and suggest that interactions between the polymorphisms in XPA/XPC Rad1/XPC and Rad51/XPD may occur.

0624

***IN VIVO* IMAGING ALLOWS DISTINCT EVALUATION OF TREATMENT RESPONSES AND DISEASE STAGES OF PATIENTS` LEUKEMIA CELLS GROWING IN MICE**

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BackgroundDistinct animal models are required to preclinically evaluate novel therapeutic approaches for treatment of acute leukemia. As a timely vision, personalized strategies might be tested using primary patients´ samples. Unfortunately, precise disease monitoring is challenging in the individualized orthotopic xenotransplantation model of acute leukemia. **Aims.** The aim was to establish bioluminescence *in vivo* imaging as novel readout technique for sensitive and reliable follow up of leukemia in mice. **Methods**Patient-derived acute lymphoblastic leukemia cells were lentivirally transduced to express the membrane-bound form of Gaussia luciferase before engraftment into mice and bioluminescence *in vivo* imaging. **Results.** Bioluminescence *in vivo* imaging enabled reliable and continuous follow-up of single mice over time. Upon detecting a single leukemia cell within more than 10,000 bone marrow cells, imaging enabled monitoring minimal residual disease, time to tumor re-growth and relapse. Imaging quantified therapy effects precisely and with low variances discriminating treatment failure from partial and complete response. As an additional advantage, imaging conveniently quantified stem cell frequencies in limiting dilution assays and visualized that vincristine impaired leukemia stem cells. **Summary and Conclusion**Taken together, the most demanding challenges in anti-cancer treatment are precisely and individually modeled in mice using Gaussia luciferase-based imaging. The model will enable translating future individualized treatment strategies from bench to bedside.

0625

CYTOGENETIC COMPLEXITY AND SUB-CLONAL ARCHITECTURE IN HIGH HYPERDIPLOID ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. High-hyperdiploidy (HHD), characterized cytogenetically by 57-61 chromosomes, is the most common recurrent abnormality in pediatric acute lymphoblastic leukemia (pALL). Controversial assumptions are available regarding the exact mechanisms underlying the formation of HHD pattern and about the sequence of various cytogenetic events (aneusomies). **Aims.** In this study, we have investigated the cytogenetic complexity of HHD pALL using multicolor interphase fluorescence in situ hybridization (MI-FISH). Clonal evolution routes and sub-clonal architecture of leukemic cell population have been revealed. **Methods.** 80 bone marrow samples withdrawn from 76 patients at the time of diagnosis or relapse (72 and 8 samples, respectively) were selected based on DNA index and/or conventional cytogenetic results. Investigating copy number alteration of eight different chromosomes (chromosomes X, 4, 6, 10, 14, 17, 18 and 21) at the single cell level using MI-FISH and automated digital microscopy, we doubled the number of targets having been previously used for this purpose. Peripheral blood cells from 19 healthy individuals served as negative controls. The study was approved by the Regional and National Ethical Committees and it was conducted in accordance with the Basic Principles of the Declaration of Helsinki. **Results.** Gains of chromosomes 6 and 14 were presented in almost all samples and a decreasing incidence of gain was observed in the following order: chromosomes X, 4, 21, 18, 10 and 17. Considering the ratio of various individual abnormalities within the abnormal cell population of each patient, gains of chromosomes X and

21 were presented at the highest level referring to their early occurrence during the formation of HHD pattern, while aneusomy of chromosome 17 proved to be the latest event. Scrutinizing the combined (eight-target) signal pattern at single cell level, a high heterogeneity has been found. The most dominant clone was presented in a range of 28 to 85 percent. The count of non-dominant, but unambiguously abnormal signal patterns varied between 25-159 and the totalized rate of cells harboring these patterns proved to be 13-70%. Comparing paired samples withdrawn from the same patient at diagnosis and relapse, it has been found that not only the appearance of new aneusomies but also an increased rate of clonal heterogeneity is associated with disease progression. **Conclusions.** In HHD pALL, extra copies of chromosomes X, 4, 6, 10, 14, 17, 18, and 21 are acquired in the vast majority of cases. While a certain level of subclonal aneuploid heterogeneity has been previously revealed by other groups, it was only assumed so far that a real cell-to-cell variation characterizes the genetic profile of HHD pALL. Comparing our cell-based results to clinical outcome of patients, we found that clonal heterogeneity may have significant prognostic value providing further stratification regarding this genetic subgroup of pALL.laszlo.pajor@kk.pte.hu

0626

T(14;18) TRANSLOCATION IN ADOLESCENTS WITH B ACUTE LYMPHOBLASTIC LEUKAEMIA/LYMPHOMA

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Background. The t(14;18)(q32;q21) translocation is a common translocation described in B cell lymphoid neoplasms It is found in 80% of follicular lymphomas and 20% of diffuse large B cell lymphomas but rarely presents in acute lymphoblastic leukaemia/lymphoma It is an independent poor prognostic predictor in adults and has not been previously described in adolescents. **Aims**. To review the progress and outcome of all paediatric cases of acute lymphoblastic leukaemia/lymphoma with t(14;18) translocation enrolled in the national MRC UKALL 2003 trial3. **Methods.** The cytogenetics coordinator was contacted to get details of all patients enrolled in the trial with t(14;18) translocation; 1 patient was identified, treated at the adolescent unit at University College London Hospital. The unit treated another patient with acute lymphoblastic lymphoma, trial-ineligible. Their notes were retrieved and reviewed in detail.4 **Results.** Patient 1 was a 14 year old male presenting with a 2 month history of increasing lethargy, muscle aches and high white cell count. Bone marrow aspirate was consistent with precursor B ALL on morphology (90% blasts) and immunophenotyping. Cytogenetic analysis showed t(14;18)(q32;q21) in 96% of interphase cells by FISH. He was commenced on UKALL 2003 Regimen B. Day 8 marrow showed 33% blasts and treatment was changed to Regimen C. At Week 15 he was found to have CNS relapse during routine lumbar puncture for intrathecal chemotherapy. He achieved a complete response with salvage chemotherapy (fludarabine, cytarabine and idarubicin - FLA-Ida) and proceeded to an etoposide/TBI/T replete sibling allograft. He was admitted 2 months post transplant with respiratory failure due to pneumonia. Despite aggressive antimicrobial therapy, intubation and ventilatory support he died at 9 months from diagnosis.Patient 2 was a 13 year old female presenting with a 2 month history of migratory polyarthritis. Whole body MRI scan showed extensive bone marrow infiltration with a large soft tissue mass in the right wrist associated with pain and swelling. Bone marrow aspirate suggested ALL/lymphoblastic lymphoma on morphology (11% blasts) but immunophenotyping did not show clonality. CT-guided biopsyof the wrist lesion confirmed precursor B acute lymphoblastic lymphoma. Cytogenetic karyotype was complex and included t(14;18)(q32;q21) in 24% of metaphases analysed by G-banding. She was also found to have CNS disease. She began treatment according to UKALL 2003 Regimen B with progressive disease; Day 8 and Day 28 bone marrow aspirates showed increase in t(14;18) initially to 33% then 44%. Repeat MRI showed persistent abnormal bone marrow signal throughout the skeleton. Salvagechemotherapy with cytarabine and etoposide (CYVE) with Rituximab was started. A complete remission was achieved with 2 cycles of chemotherapy and she underwent a Cyclophosphamide/TBI/Campath matched unrelated donor allograft. Within 6 weeks her white cell count increased, and peripheral blood immunophenotyping confirmed relapsed disease. She died at home within 6 months of diagnosis. **Summary and Conclusions.** Precursor B acute lymphoblastic leukaemia/lymphoma with t(14;18)(q32;31) translocation is a poor prognostic indicator with an aggressive and fatal course in adult patients Both patients described here suggest that this disease in the adolescent population has a similarly poor outcome

Acute myeloid leukemia - Biology 2

0627

TRIB2 GENE DYSREGULATION IS ASSOCIATED WITH ADULT MINIMALLY DIFFERENTIATED ACUTE MYELOID LEUKEMIA

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Background. Tribbles homolog gene 2 (TRIB2) is a pseudokinase gene belonging to a three member gene family. Tribbles gene was first identified in Drosophila melanogaster where is involved in the regulation of morphogenesis and mitosis. Likewise, mammalian homologous genes of Tribbles (TRIB1, TRIB2, TRIB3) promote the degradation of specific transcription factors and interact with several cell signaling mediators and modulators. Recently it has been demonstrated that TRIB2 was able to induce AML in bone marrow transplanted mice by inducing the proteasome-dependent degradation of C/EBPα. Moreover, gene expression profiles data from AML patients revealed that patients carrying C/EBPα mutations clustered with those patients with TRIB2 up-regulated. **Aims.** To test the hypothesis that the TRIB2 expression was associated to the differentiation degree in AML, we evaluated several cases based on FAB cytotype. **Patients and Methods.** We performed quantitative Real Time-PCR (qRT-PCR) analysis on bone marrow aspirate samples of AML patients (15 AML-M0, 14 AML-M2, 5 AML-M3 and 3 M5b) to measure the expression level of TRIB2. Healthy bone marrows were used as reference samples. The qRT-PCR was conduct using SYBR green chemistry, specific primers for TRIB2 transcript and two housekeeping genes (B2M and IPO8) previously tested. Each AML sample was tested by conventional cytogenetic analysis and by FISH using a TRIB2 specific probe. We performed, also, the mutational analysis of the TRIB2 coding sequence and the methylation analysis of a CpG island located in the TRIB2 promoter region through the methylation sensitive restriction enzymes (MSRE) and qPCR. **Results.** qRT-PCR experiments revealed that TRIB2 expression was higher (from 3 to 20 fold) in AML-M0 respect to the AML-M2 (p= 0.02), AML-M3 (p=0.01), AML-M5 (p=0.006) FAB subtypes, and references (p=0.003), respectively. Therefore qRT-PCR displayed a progressive decreasing TRIB2 expression across FAB subtypes. To disclose the reasons of such misregulation we performed further analysis. Conventional cytogenetic and FISH analysis did not show any kind of rearrangement involving TRIB2 gene as found in other cancers with TRIB2 up-regulated, and mutational analysis excluded the occurrence of activating mutations. MSRE experiments showed an higher methylation degree in a CpG island, located in the TRIB2 promoter region in AML-M0 cases when compared to the others FAB subtypes; moreover, methylation degree was significantly correlated to the TRIB2 expression (r = 0.9; p = 0.0002). **Conclusions.** Here we show that the TRIB2 expression correlate with the differentiation degree of leukemic cells in AML, and it is epigenetically regulated. In our cases the TRIB2 misregulation was not due neither to gene amplification nor to activating mutations. Our data reveal that TRIB2 gene promoter is differentially methylated according to the FAB subtypes. Usually, the methylation causes gene silencing but we have found that the methylation of TRIB2 promoter region was associated to the overexpression of the gene.Our hypothesis is that the methylation could inhibit binding of some unknown transcriptional repressor as already seen for hTERT gene in others tumors cells.

0628

THERAPY RESISTANT AML STEM CELLS CHARACTERIZED BY IMMUNOPHENOTYPE (CD34/CD38) AND FUNCTION (DRUG EFFLUX)

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Background. Despite intensive therapy 40% of acute myeloid leukemia (AML) patients relapse after remission. Relapse is thought to originate from leukemia initiating cells/stem cells (LSC) most often described as CD34+CD38-. However, the side population (SP), which is based on activity of drug efflux pump activity, and thereby possibly linked with therapy resistance, is also enriched in LSC. **Aims.** To determine the relationship between the CD34+CD38- defined LSC and those based on SP analysis. **Methods.** We analysed CD34 and CD38 expression within SP and non-SP (NSP) compartments of 6 CD34 positive AML samples and 7 CD34 negative samples (only molecularly normal CD34 cells) Populations were defined as putative AML or normal based on

immunophenotypic aberrancies (van Rhenen, Leukemia 2007, Blood 2007; Moshaver Stem Cells 2008). In 2 CD34- and 5 CD34+ samples, molecular aberrancies were determined (after cell sorting) to distinguish between AML and normal. In 2 CD34- and 3 CD34+ samples SP/NSP fractions were tested for stem cell activity in the liquid culture colony assay. **Results.** As expected, the CD34+CD38- and CD34+CD38+ defined populations of the 7 CD34 negative cases, were immunophenotypically normal (lack of the markers CLL-1, CD56, CD7). In 2 cases (NPM1-mut and FLT3-ITD), all CD34+CD38- and CD34+CD38+ SP and NSP sub-populations were molecularly normal. CD34-CD38+ made up almost all (> 90%) CD34- cells, both in SP and NSP, and were largely (52.0- 99.5%) leukemic.For CD34 negative AML, both leukemic and normal clonogenic output (colonies per million input cells) was highest for the SP fraction (SP AML vs NSP AML: 16,000 vs 20; SP normal vs NSP normal: 8,000 vs 0).In the 3 CD34 positive AML with low CD34 percentages (0.1%, 8.7%, 10.3%) that were molecularly characterized (2 FLT3-ITD, 1 NPM1-mut) the CD34+CD38- within the SP population were molecularly normal. With higher CD34 percentages (17%, 55%, 93%) a larger part (16%, 43%, 85%) of the SP CD34+CD38- cells were leukemic: 2 both molecularly (1 NPM1-mut, 1FLT3-ITD) and immunophenotypically aberrant; 1 immunophenotypically aberrant) In the NSP fraction of all 6 CD34+ patients, the CD34+CD38- cells were largely (60-100% leukemic (3 FLT3-ITD, 2 NPM1-mut and in addition all 6 were immunophenotypically aberrant). Also for CD34+CD38+ cells, with increasing CD34 percentage, the contribution of leukemic cells within SP increased (23%, 93%, 100%). In the NSP fraction the CD34+CD38+ were largely (41-100%) leukemic. CD34-CD38+ cells were largely (50-100%) leukemic in both SP and NSP. Again, for CD34 positive AML, the clonogenic output was highest for the SP fraction (SP AML vs NSP AML: 4,900 vs 7; SP normal vs NSP normal: 2,300 vs 0). **Conclusions.** These results indicate that SP analysis can help to define small fractions of LSC that are highly clonogenic in both CD34 positive and negative AML. Since the frequency of CD34+CD38- LSC has strong prognostic impact *(Terwijn, ASH 2010;759)*, and the SP population is defined by high drug efflux ability, it must be considered that it may well be the CD34+CD38- SP fraction that preferentially survives therapy.

0629

WILMS' TUMOR 1 PROTEIN EXPRESSION IS DOWNREGULATED BY MICRORNA-132

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Background. Apart from mutations in specific genes, aberrant expression of crucial genes is thought to contribute to leukemogenesis. Apart from transcription factors, microRNAs are important regulators of gene expression, both at the mRNA as well as the protein level. MicroRNAs contribute to the regulation of hematopoiesis via targeting of transcription factors or genes involved in cell cycle regulation and proliferation. Several microRNAs were shown to be associated with hematological malignancies, but information on the relevant target genes that are regulated by these microRNAs is sparce. **Aims.** We performed microRNA expression profiling to identify specific microRNAs which target genes involved in myeloid differentiation. **Methods.** The expression of 157 mature microRNAs was studied during granulocytic differentiation (induced by all-*trans* retinoic acid (ATRA)) in the acute promyelocytic leukemia cell line NB4 using stem-loop RT-PCR Taqman® microRNA assays (Applied Biosystems). Identification of candidate target genes of specific miRNAs was performed using four microRNA target prediction programs (DIANA-microT, miRanda, PicTar, TargetScanS). **Results**. We identified miR-132 as the most strongly induced microRNA during ATRA-induced differentiation in NB4 cells (491-fold). Induction of miR-132 during granulocytic differentiation was also observed in the non-APL cell line HL60 (31-fold), as well as in leukemic primary cells (9-fold, N=8) and normal primary cells (15 fold, N=5) which were obtained after informed consent in accordance with the Declaration of Helsinki and institutional review board approval. These data indicate that granulocytic differentiation is associated with induction of miR-132.We identified Wilms' Tumor 1 (*WT1*) as a putative target of miR-132 based on the presence of a conserved potential miR-132 recognition element (MRE) in the 3'UTR of *WT1*. The *WT1* gene encodes for a zinc-finger transcription factor which plays an important role in normal as well as malignant hematopoiesis. In myeloid malignancies, deregulation of WT1 is frequently observed. So far, the regulation of *WT1* expression has mainly been studied at the transcriptional level. The mechanisms of post-transcriptional regulation of *WT1* expression are largely unknown. We studied whether miR-132 can regulate *WT1* expression.To confirm the predicted interaction of miR-132 with the 3'UTR of *WT1*, luciferase reporter assays were performed. If the wild-type 3'UTR of *WT1* was present downstream of the firefly luciferase gene, co-expression of miR-132 resulted in a significant reduction of the firefly luciferase signal (mean reduction: 33±3%, N=3). Repression of the luciferase expression by miR-132 was lost if complementarity between the *WT1* 3'UTR and miR-132 was disturbed by mutations. To determine whether endogenous *WT1* expression is indeed regulated by miR-132, the effect of ectopic miR-132 expression on *WT1* mRNA and protein expression was studied. Endogenous *WT1* mRNA expression was not affected by ectopic expression of miR-132. However, endogeneous *WT1* protein levels were significantly lower in miR-132 transduced NB4 cells compared to empty vector transduced NB4 cells. **Summary and Conclusions.** We show that protein translation of *WT1* is repressed by miR-132 via a specific miR-132 recognition element located in the 3'UTR of *WT1*. We conclude that miR-132 functions as a translational repressor of *WT1*.

0630

THE NOVEL TYROSINE KINASE INHIBITOR PONATINIB (AP24534) MAY OVERCOME RESISTANCE OF FLT3-ITD HARBOURING THE PREVIOUSLY REFRACTORY F691I MUTATION IN VITRO

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Background. Molecular aberrations of the *fms*-like tyrosine kinase 3 (FLT3) represent the most frequent mutations that confer an unfavourable prognosis in acute myeloid leukemia (AML). Particularly, a ligand-independent autophosphorylation of FLT3 by internal tandem duplications (ITD) defines a promising target for therapeutic approaches using FLT3 tyrosine kinase inhibitors (TKI). However, distinct additional point mutations within the FLT3 tyrosine kinase domain (TKD) have been identified as one major mechanism to mediate secondary resistance towards FLT3 inhibitors. Recently, pharmacological research revealed ponatinib (AP24534) as an orally active TKI with inhibitory activity against tyrosine kinases including FLT3. **Aims and Methods.** In this study, we intended to evaluate the efficacy of the recently characterized FLT3-TKI ponatinib to overcome TKI resistance in murine Ba/F3 cells transfected with FLT3-ITD containing additional point mutations of the FLT3-TKD, notably the multi-resistant F691I mutation. **Results.** We can demonstrate that ponatinib potently induces apoptosis not only in the parental FLT3-ITD cell line but also in all stably transfected mutants harbouring the additional FLT3-TKD point mutations N676D, F691I, or G697R. Importantly, these observations correlate with a strong inhibition of FLT3-ITD and its downstream signalling pathways (STAT5, AKT, and ERK1/2) upon ponatinib treatment as determined by phospho-specific immunoblot analysis.Furthermore, ponatinib mediates a dose-dependent down-regulation of the important anti-apoptotic protein Mcl-1 in the FLT3-ITD harbouring cell line while a significant lower expression and an impaired regulation of Mcl-1 was detectable in Ba/F3-ITD-F691I cells by cytometry and Western blot analysis. **Conclusions.** Taken together, these results approve that ponatinib represents a promising FLT3-TKI *in vitro*. Therefore, clinical trials are warranted to further improve the therapeutic options for FLT3-ITD-positive AML patients. Moreover, the targeted therapy of FLT3-ITD-positive AML with ponatinib might be associated with a lower frequency of secondary resistance caused by acquired FLT3-TKD mutations.

0631

NOVEL SMALL MOLECULE INHIBITORS OF THE P53- MDM2 /MDM4 INTERACTION FOR INDUCTION OF APOPTOSIS IN AML

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A promising new approach in cancer treatment is the inhibition of murine double minute 2 and 4 (MDM2/MDM4) which is a negative regulator of p53. In neoplasia with unmutated p53 the inactivation of MDM2/4 promotes apoptosis and growth arrest. A derivative of the first described p53/MDM2 antagonist Nutlin-3 has already entered clinical phase I / II studies, although recent *ex vivo* data also indicate that Nutlin-3 is able to induce DNA damage and promotes tumor cell resistance. We investigated a novel set of substances and their ability to

antagonize the protein-protein interaction between p53 and MDM2/4.The compounds described here, belonging to the YH-compound family, were synthesized according to the proposed structure by ANCHOR.QUERY based design and modelling in the p53/MDM2 protein structure. This YH-compound family consists of an acyclic Ugi four component reaction scaffold. The reaction yielded a mixture of enantiomers that could be easily separated by chiral HPLC. The initial screening by nuclear magnetic resonance (NMR) and fluorescence polarisation (FP) revealed several p53/MMDM2 antagonists. Further structure based optimisation led to potent and selective drug-like lead structures which were confirmed by co-crystal structures.To evaluate the biological effects of our compounds we analyzed four AML cell lines in which in general p53 mutations are rare events. The initial experiments were performed in OCI-AML-3 and MOLM-13 which were both confirmed to be wt-p53 and HL-60 and NB4 with deleted or mutated p53 (mt-p53). We determined the EC_{50} values of our substances by measuring the turnover of WST-1 to Formazan, depending on the cell metabolism. We investigated the influence on the metabolic activity of the wt-p53 and mt-p53 cells caused by treatment with our substances in different concentrations for 48 h. Notably, the compound YH239 showed an EC_{50} of 14 µM in MOLM-13 cells. To get further evidence of efficacy of the lead compound YH239 we analysed the influence on the cell cycle after 24 h treatment. A relative increase of 23% compared to untreated cells in the subG1 phase was observed. Furthermore, we measured the cell numbers and cell viability up to 72 h of treatment. In the wt-p53 MOLM-13 cell line a growth reduction of 65% was measured compared to no effect in the p53 deleted HL-60 cell line. Induction of apoptosis was analyzed after 72 h treatment with 20 µM YH239 by Annexin-V and propidium iodide (PI) staining. We noticed 86% of specific apoptotic cells in wt-p53 MOLM-13 cell line compared to 2 % in p53 deleted HL-60 cell line relative to the untreated control. A measurement of caspase 3/7 activity upon treatment (6 h and 12 h) with our substances indeed revealed an increased signal. Comparing both enantiomers YH239 A and B we found that YH239B is the more active one. In conclusion YH239 shows high efficacy for induction of apoptosis and cell growth arrest in wt-p53 cells. Further investigations in primary AML cells are under way.

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MICRORNA EXPRESSION PROFILING OF HEMATOPOIETIC AND LEUKEMIC STEM CELLS IN ACUTE MYELOID LEUKEMIA

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Background. Only a minority of cells in acute myeloid leukemia (AML) is responsible for leukemia growth and maintenance. These leukemia-initiating cells share cell surface markers with normal hematopoietic stem cells (HSCs) and have features of both self-renewal and differentiation which have given them the name "leukemic stem cells" (LSCs). Although 60-80% of AML patients achieve complete remission after chemotherapy, many patients experience a relapse which is thought to be caused by the survival of LSCs after chemotherapy. Eradication of LSCs is therefore necessary to cure AML patients.Both normal HSCs and LSCs co-exist in the bone marrow (BM) of AML patients and success of anti-LSC therapy relies on eradication of LSCs while sparing HSCs. For the development of these LSC-specific therapies the identification of molecules that are differentially expressed between normal and malignant stem cells is important. MicroRNAs (miRNA) are small non-coding RNAs which regulate gene expression at the post-transcriptional level by targeting mRNAs. These small RNAs are promising therapeutic targets since modulation of a single miRNA may affect many pathways simultaneously. **Aims.** Our aim is the development of novel LSC specific miRNA-based therapeutic strategies by identification of miRNAs differentially expressed and functioning in LSCs and HSCs. **Methods.** We have identified several phenotypic differences between LSCs and HSCs that reside within the CD34+CD38- compartment of the AML BM. These include aberrant expression of CD34, CD45, CLL-1, lineage markers, scatter properties, and recently the activity of aldehyde dehydrogenases. Stem cells with high aldehyde dehydrogenase activity have no molecular and immunophenotypical aberrancies suggesting a normal phenotype whereas stem cells with low aldehyde dehydrogenase activity harbor leukemia associated aberrancies. This allowed the identification and purification of both LSCs and HSCs from AML patients and gave us the opportunity to identify miRNAs differentially expressed between LSCs and HSCs and between LSCs and leukemic progenitors all derived from the same AML BM (n=6). **Results.** Microarray analyses revealed the differential expression of miR-21, miR-181a/b, miR-551b, miR-29b and miR-125b in LSCs compared to HSCs. MiRNAs with differential expression between LSCs and leukemic progenitors were miR-126/miR-126*, miR-335, miR-146a and miR-1260. We have confirmed the expression patterns of several of the identified miRNAs by qRT-PCR. **Conclusions.** In conclusion, we have identified several miRNAs that are differentially expressed between LSCs and HSCs and LSCs and leukemic progenitors. These miRNAs might characterize and potentially maintain AML LSCs through their effects on proliferation, apoptosis and differentiation. In the future these miRNAs may be used as valuable anti-leukemic miRNA-based therapeutics.

0633

MUTATIONS OF THE SPLICEOSOME MACHINERY AND OF EPIGENETIC ENZYMES ARE RARE IN THERAPY-RELATED MYELOID NEOPLASMS

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Background Therapy-related neoplasms (t-MN), including myelodysplastic syndromes and acute myeloid leukemia (AML), are an increasingly recognized complication in patients previously treated with radiotherapy and/or chemotherapy for a cancer or autoimmune disease. t-MN display a high incidence of monosomal and complex karyotypes, *p53* mutations, and gene-specific promoter methylation, but some other mutations that are common in AML, are usually rare, as mutations in the genes FLT3, NPM1, CEBPA, and TET2. **Aims.** We studied the incidence of the recently described mutations in genes coding enzymes involved in epigenetic pathways (IDH1 R132, IDH2 R140 and R172, DNMT3A R882), and of genes of the spliceosome machinery (U2AF1, SF3B1 exons 13-14 and 15-16) in 101 patients with a therapy-related MN. We also analyzed the prevalence of methylene-tetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms (SNP), compared to age and sex-matched controls. **Methods.** MTHFR SNP were studied using PCR-RFLP, while analysis of all other genes was performed on genomic DNA by Sanger sequencing (ABI PRISM 3130 Genetic Analyzer; Applied Biosystems). **Results.** There were 56 females and 55 males, with a median age of 66 years (range 16-88 years).The homozygous 1298A>C MTHFR variant was more frequent in t-MN (7/49 patients versus 5/110 controls, p=0.03). In this line, aplotype frequency of the CC vs CA and TA vs CA was significantly higher in t-MN compared to controls (p<0.005 for both). There were 4 mutations in the IDH1 gene at position R132 (4/96 patients, 4.2%, 3 R132C, 1 R132H),6 inthe IDH2 gene, (2 R172K and 4 R140, 6/94 patients, 6.4%). IDH1 and IDH2 mutations were mutually exclusive. DNMT3A was mutated at R882 in 5 of 95 patients (5.3%, 4 R882H and 1 R882C).The recurring K700 alteration in exons 15-16 of the SF3B1 gene was detected in 3 of 94 t-MN patients (K700E, 3.2%), so far we did not find any Exon 13-14 mutations. A concurrent R140Q IDH2 mutation and R882H DNMT3A mutation was observed in 2 patients.Looking at patient characteristics, there were no associations between the incidence of mutations and the proportion of bone marrow blasts or type of t-MN. IDH1 and IDH2 mutations were significantly more frequent in t-MN secondary to a myeloproliferative neoplasm (MPL) (5 of 14, versus 5 of 49 with a previous solid tumor, versus none of the 24 patients with a previous lymphoproliferative disease, p: 0.002). Two of two patients with available DNA from the MPL and the subsequent t-MN had the same pattern of mutations before and after evolution (1 mutated, 1 Wild Type). The pattern of mutations was similar in peripheral blood -mononuclear cells and CD34+ cells selected at the time of leukemic evolution in 3 patients.IDH mutations confirm that disease evolution plays a fundamental role in AML secondary to MPL. **Conclusions.** IDH 1/2, DNMT3A and SF3B1 mutations are rare in MDS/AML secondary to chemo- or radiotherapy for solid tumors or other hematological malignancies. In these diseases mutations of other pathways and aberrant karyotype may play a dominant role.

0634

THE EXPRESSION OF THE EGF-TM7 RECEPTOR CD97 IS HIGHER IN CD34-NEGATIVE AND NPM1/FLT3-ITD MUTATED AML

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Background. With an incidence of 25% the internal tandem duplication (ITD) of the receptor tyrosine kinase FLT3 represents one of the most common mutations in patients with AML which results in constitutive aberrant activation of FLT3 and increased proliferation of leukemic progenitors.CD97 is a member of the EGF-TM7 family of adhesion receptors and is broadly expressed in hematopoietic cells. In carcinomas of epithelial origin CD97 showed increased expression which correlated in some cases with a higher tumor grade and poor prognosis. **Aims.** So far, nothing is known about the expression of CD97 in AML bone marrow (BM) which was investigated in the present study. **Methods.** We studied 116 samples from patients with *de novo* acute leukemia, comprising AML M0-2 (n=73), AML M3 (n=7), AML M4/5 (n=31) and c-ALL (n=5). A 4-color immunophenotypic measurement was performed on a FACS Canto II using the following antibodies: CD14, CD2 and CD7 FITC, CD13 PE, CD34 Per-

Cp5.5, CD117 and CD56 PE-cy7, CD97 and CD33 APC, HLA DR APC-H7, CD45 V500. FLT3-ITD mutations and NPM1-mutations were detected as reported in detail previously (Thiede C et al. Blood 2002; Thiede C et al. Blood 2006).The human AML cell line MV4-11 which carries a homozygous FLT3-ITD mutation and normal CD34+ hematopoietic stem cells (HSC) were exposed to different concentrations of the protein kinase inhibitor PKC412 (Midostaurin). After 24 hours the CD97 expression was analyzed by flow cytometry and the migration capacity was tested in a transwell chamber assay. **Results.** Compared to BM blasts of healthy donors (n=10), we detected significant higher CD97 expression levels (mean fluorescence intensity, MFI) in 50 AML samples (42%), which is 31.5% of M0-2, 100% of M3 and 45% of M4/5, and 80% of the c-ALL, respectively. Patients with a CD97 expression above the mean on leukemic blasts showed also increased expression of the molecule within the residual granulo- and monopoiesis. Of note, higher CD97 expression was accompanied by a significantly higher BM blast count (75% vs. 53%, p<0.001). Interestingly, elevated CD97 expression was associated with mutations in NPM1 (46% vs. 18%, p=0.003) and FLT3 genes (39% vs. 7%, p<0001) as well as lower CD34 expression (46% vs. 81%, p<0.001). Furthermore, no AML1/ETO or CBFb/MYH11 fusion genes were detectable in CD97+ AML vs. 6% in CD97- AML. *In vitro*, we detected higher CD97 expression levels in MV4-11 AML cells in comparison to CD34+ HSC (MFI: 138 vs. 45.7). Treatment with PKC412 resulted in a decrease to 40.4% of CD97 expression in MV4-11 cells whereas it had no influence in CD34+ HSC. The lower CD97 expression levels correlated with inhibition of the spontaneous migratory capacity of the AML cell line. **Conclusions.** We present first evidence of higher CD97 expression in mFLT3/mNPM1 AML cells which might provide a new diagnostic and perhaps therapeutic target. The possible impact of CD97 expression on AML biology and clinical outcome should be evaluated in a larger patient cohort.

0635

COSTIMULATORY MOLECULE B7-H3 IN ACUTE LEUKEMIA: EXPRESSION CHARACTERISTICS AND PROGNOSTIC SIGNIFICANCE

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Background. B7-H3 is a recently identified costimulatorymolecule belonging to B7 family that has been implicated as a novel modulator of antitumor response. Many researches on B7-H3 expression and clinical significance of various human cancers were studied but not in hematopoietic malignancies.2. **Aims.** To contribute to an understanding of its role in acute leukemia (AL), we analyzed B7-H3 at protein levels by flow cytometry (FCM) and mRNA by RT-PCR in 134 patients with AL and 12 age- and sex- matched healthy controls. **Methods.** Positive expression was defined as ≥10% leukemia cells co-expressing B7-H3 molecule comparing with IgG mAb isotype control. Correlations between B7-H3 expression and clinico-biological characteristics were calculated for prognostic-prediction. Among all the patients, 101 acute myeloid leukemia (AML) and 33 acute lymphocytic leukemia (ALL) were included, with male/female ratio of 1.2:1 and median age of 43 years. B7-H3 was detected on surface of blast cells of ALs by FCM. RT-PCR confirmed the products at mRNA level within same samples. **Results.** According to our definition, 44.8% AL cases were B7-H3 positive expression and none was in healthy controls. Heterogeneously, positive rate and expression range of B7-H3 in AL subtypes were as follows: M1 42% (1.3~43.0%), M2 50% (0.5~74.8%), M3 22% (2.1~32.3%), M4 44% (1.2~43.1%), M5 36% (1.1~54.3%), B-ALL 54% (1.1~66.5%), T-ALL 20% (1.9~23.9%). No statistical special distribution was found in any subtype. From a clinico-biological standpoint, no significant relationship was observed between B7-H3 expression and sex, age, percentage of blast cells or white blood cell counts (WBC). However, B7-H3 had a significant higher expression in $CD34^+$ cases versus $CD34^-$ cases (P=0.018) and poor- versus good- or intermediate- risk karyotypes whether in AML or ALL patients (P=0.000). Likewise, the expression of B7-H3 was statistically relevant in predicting disease progression (P=0.001) and a shorter life survival (P=0.005). **Summary and Conclusions.** In summary, this study observed for the first time that B7-H3 also expressed on membranes of leukemia cells in ALs. B7-H3 expression correlated with progenitor marker CD34 and poor risk karyotype but not with other prognostic factors. Furthermore, in AL patients, B7-H3 expression predicted a shorter PFS and OS. Our results suggest that B7-H3 expression has prognostic significance.

0636

DIFFERENTIAL EFFECT OF CYTOTOXIC MAINTENANCE THERAPY ON T CELLS, TREGS AND NK CELLS IN AML PATIENTS

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The success of immunotherapies is hampered by negative regulatory mechanisms that inhibit anti-leukemic T and NK cell function. A promising approach to overcome immune evasion is the combination of immunotherapy with cytotoxic chemotherapy. Within the AML-CG study group, we regularly apply cytarabine in an alternating combination with daunorubicin, 6-thioguanine and cyclophosphamide every 28 days as a maintenance postremission therapy in AML patients not eligible for stem cell transplantation.Assessing the immune status of patients prior to and during maintenance therapy, we aimed at defining a potentially beneficial point of time for active immunotherapies, e.g. dendritic cell vaccination.We analyzed PBMC subpopulations ($CD3^+CD8^+$ cytotoxic T cells, $CD3^+CD4^+$ T helper (Th) cells, $CD4^+CD25^{hi}CD127^{lo}FoxP3^+$ regulatory T cells (Tregs), and $CD3^-CD16^+CD56^+$ natural killer (NK) cells) in 10 AML patients throughout early maintenance therapy cycles by flow cytometry. In addition, Tregs were quantified by a DNA methylation assay in 3 AML patients. In order to test the proliferative capacity of Th cells, CFSE assays with PHA as stimulus were performed in 3 patients. T cell polarization was analyzed by determination of secreted cytokines using a cytometric bead array after IL-2 stimulation. NK cell functionality was assessed in 4 patients using CD69 upregulation after stimulation with IL-2. The resulting data was compared to a total of 15 healthy donors.The absolute Th cell counts, absolute numbers of Tregs and absolute NK cell counts were significantly lower in AML patients prior to the start of maintenance therapy compared to healthy donors. In the course of maintenance therapy, several dynamics were observed: Total leukocyte numbers decreased, with the nadir around 1 G/l on day 21. The proportion of Th cells temporarily increased throughout the course of therapy. So did the fraction of Tregs, which more than doubled throughout therapy, explaining part of the relative increase in $CD4^+$ T cells. In contrast, relative numbers of NK cells declined in the course of maintenance therapy. Functionally, proliferative response of Th cells from AML patients was substantially reduced two weeks after the start of therapy. When comparing cytokine secretion after stimulation with PHA, we found that the Th cells isolated from AML patients tended to secrete higher concentrations of IL-4 and IL-17, but lesser amounts of IFN-γ. The activation capacity of NK cells was generally reduced in AML patients pretherapy relative to healthy donors.In our ongoing study of AML patients, we found that T and NK cell subsets after intense polychemotherapy significantly differed from those of healthy donors. Prior to the start of maintenance chemotherapy, we prominently saw a diminished number and function of NK cells. This is in accordance with previous reports on NK cell dysfunction in AML patients. In the course of maintenance therapy, we observed a significant relative increase in Tregs and a decrease in the functional response of Th cells. These results urge to intensively monitor T cell populations and functionality during chemotherapy and immunotherapy to be able to establish synergistic combinations of chemotherapy and immunotherapy.

0637

THE ROLE OF AUTOPHAGY IN ACUTE MYELOID LEUKAEMIC CELL DIFFERENTIATION

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Background and Aims. Autophagy is a degradative cellular process in which aged, damaged or redundant proteins and organelles are engulfed within membrane-bound structures known as autophagosomes and metabolised by lysosomes. As well as being activated in times of cellular stress, autophagy has recently been proposed as a key player in cellular remodelling and differentiation. As differentiation block is a hallmark of Acute Myeloid Leukaemia (AML), we set out to characterize the involvement of autophagy in myeloid leukaemic cells undergoing pharmacologically induced differentiation. Morphologically autophagy is characterized by the accumulation of vesicles within the cell cytoplasm. Gabba-aminobutyric-acid-receptor-associated-protein (GABARAP) is a cytoplasmic protein that is conjugated on autophagy activation and recruited to these vesicles. **Methods and Results.** We treated NB4 cells - a model for Acute Promyelocytic Leukaemia (APL) carrying the PML-RARA translocation, with all-*trans*-retinoic acid (ATRA) (1uM) for 6 days. As expected, differentia-

tion along a granulocytic lineage was observed morphologically on Prodiff staining of cytospun cells. This was corroborated by a proliferation arrest as measured by daily viable cell counts (Chemometec Nucleocounter system) and confirmed by flow cytometric detection of the surface markers of early differentiation - CD11b and CD14.Microscopically, we observed a significantly increased number of cytoplasmic vesicles in ATRA-treated cells after 48 hours as compared with untreated controls. The presence of these vesicles preceded the characteristic cellular changes seen with granulocytic differentiation and we enumerated a progressive increase in vesicle quantity as differentiation advanced. We carried out immunoflourescence analysis of GABARAP in NB4 cells undergoing differentiation and observed a shift from diffuse cytoplasmic staining on Day 1 to a definite punctate pattern on Day 6 consistent with incorporation into autophagic vesicles. GABARAP was also seen to surround ring-shaped structures suggesting the formation of larger autophagic vacuoles. Western Blotting performed on protein lysates prepared from treated and control cell populations to detect Beclin-1 - a well-characterized protein involved in the initiation of autophagosome formation, revealed an increase in protein level from Day 3 of ATRA treatment as compared to untreated controls, suggestive of BECN1 gene up-regulation during the differentiation process. **Conclusions.** Autophagy plays a significant role in the myeloid differentiation of APL cells. Pharmacological induction of autophagy may have the potential to induce differentiation in other forms of AML. We are currently testing this hypothesis on AML patient samples.

0638

REDUCED RISK OF THERAPY-RELATED ACUTE MYELOID LEUKEMIA IN CARRIERS OF THE BCL2-LIKE-10-LEU21ARG VARIANT

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Background. Proteins belonging to the Bcl-2 family are key regulators of apoptosis and have been shown to mediate survival and cell death processes. Bcl2L10 (also named Diva, Boo or Bcl-B) is a member of the Bcl-2 family characterized by an ambiguous function. Specifically, previous independent reports indicated that Bcl2L10 can have both pro- or antiapoptotic functions and it can influence the transit between quiescence and proliferation. The aberrant regulation of apoptosis and cell cycle have been directly linked to many diseases and is one of the hallmarks of cancer.We have previously demonstrated that Bcl2L10 is transcriptionally repressed by promoter hypermethylation and that its overexpression correlates with apoptosis and growth inhibition of the HL60 cell line. Moreover, patients affected by *de novo* and therapy-related acute myeloid leukemia (AML) were more frequently methylated at Bcl2L10 compared to controls. High levels of Bcl2l10 methylation were a negative prognostic factor for 5-azacytidine treatment in patients affected by a higher-risk myelodisplastic syndrome (MDS). **Aims.** We performed a case-control study to test the prevalence of the polymorphic variant of the Bcl2L10 gene (Bcl2L10 Leu21Arg; rs2231292) as risk factor for *de novo* and therapy-related AML/MDS.

Genotype	Controls	MDS	AML	t-MDS/AML	Statistical Analysis*	
	n (%)	n (%)	n (%)	n (%)	OD (95% CI)*	p-Value*
Bcl2L10-Leu21Arg	162	92	75	84		
Leu/Leu	57 (35.19)	39 (42.39)	24 (32.00)	43 (51.19)	1.00 (Ref)	
Leu/Arg	85 (52.47)	39 (42.39)	41 (54.67)	33 (39.29)	**0.51 (0.28-0.94)**	**0.029**
Arg/Arg	20 (12.34)	14 (15.22)	10 (13.33)	8 (9.52)	0.53 (0.19-1.43)	0.246
Leu/Arg and Arg/Arg	105 (64.81)	53 (57.61)	51 (68.00)	41 (48.81)	**0.52 (0.29-0.92)**	**0.022**

**The statistical analysis refers to t-MDS/AML patients vs controls. Significant differences between t-MDS/AML and controls are in bold.*

Figure 1.

Methods. Bone marrow or peripheral blood samples were obtained from 75 *de novo* AML, 92 *de novo* MDS and 84 therapy-related MDS/AML patients, diagnosed according to the WHO classification. Control peripheral blood samples were obtained from 162 Caucasians with a negative history for previous malignancies. Informed consent was obtained according to institutional guidelines. We established a PCR-RFLP technique to detect the Bcl2L10 Leu21Arg polymorphic variant. Hardy-Weinberg equilibrium was calculated for each population and Yates corrected test was used to calculate differences in genotypes population. Odds ratios with 95% confidence intervals were also calculated. **Results.** This is the first report on the frequency of the Bcl2L10 Leu21Arg SNP in myeloid malignancies. Frequencies of the polymorphic variant Leu21Arg of Bcl2L10 enzyme were similar in *de novo* AML, MDS patients and controls. On the other hand, the Bcl2L10 variant was less frequent in therapy-related MDS/AML patients compared to controls (O.R., 0.52; 95% C.I., 0.29-0.92; p=0.022), resulting in a reduced risk for therapy-related AML/MDS (table 1). The same was true when comparing therapy-related MDS/AML to *de novo* AML (O.R., 0.45; 95% C.I., 0.22-0.90; p=0.022), but not comparing to *de novo* MDS. Moreover, stratifying the analysis, the frequency of Bcl2L10 variant was lower in therapy-related AML than in *de novo* AML (O.R., 0.36; 95% C.I., 0.16-0.83; p=0.013), whereas no differences were found between *de novo* and therapy-related MDS.No significant associations were found between enzymatic polymorphisms and other patients' characteristics, including sex, age, cytogenetics, and therapy of the primary tumor. **Summary and Conclusions.** Bcl2L10 Leu21Arg variant is less frequent in therapy-related AML, compared to *de novo* AML and controls, probably playing a protective role against the development of secondary myeloid neoplasms.

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ASSOCIATION OF CYP2B6 G516T POLYMORPHISM WITH THE SUSCEPTIBILITY OF *de novo* ACUTE MYELOGENOUS LEUKEMIA

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Background. Acute myeloid leukemia (AML) is a heterogeneous disease with well-known clinical and pathological aspects. Nevertheless, the genetic etiology of AML which include gene mutations and chromosomal aberrations is largely unknown. Altered forms of genes that differ by a single nucleotide base-pair polymorphisms (SNPs) have been shown to predispose individuals to AML development. Interindividual differences based on detoxification genes polymorphisms may contribute to the AML susceptibility. Cytochrome P450 (CYP) enzymes are involved in the metabolism of many endogen and exogenous xenobiotics that act as precarcinogens. *CYP2B6, a* phase I metabolic enzyme, blocks the transformation of precarcinogens to their biologically active forms that provoke chromosomal instability and cancer. *CYP2B6* $G^{516}T$ SNP change the aminoacid sequence (Gln172His), resulting in enzymatic inactivation. Thus, individuals homozygous for the mutant allele (T/T) completely lack *CYP2B6* activity, whereas heterozygotes (G/T) present decreased enzymatic activity. **Aims.** We performed a case-control study in a series of AML patients to investigate the potential relation between the *CYP2B6* G/T and T/T variant genotypes and the risk of *de novo* AML. We also compared the genotypic frequencies in AML patients in respect to chromosome abnormalities, FAB classification and clinical characteristics. **Methods.** The *CYP2B6* $G^{516}T$ genotyping was performed on 132 *de novo* AML patients at diagnosis and 168 sex and age matched healthy controls using a PCR-RFLP assay. Unstimulated bone marrow cells were used for karyotypic analysis and karyotypes were described according to ISCN. Statistical analysis was performed using Chi-square test and $P<0.05$ was considered to be statistically significant. **Results.** Karyotypic analysis and *CYP2B6* genotyping were successfully performed in all 132 AML patients at diagnosis. Among them, 91 (68.9%) showed clonal karyotypic abnormalities. The genotypic distribution in patients and healthy controls groups showed: homozygous wild type G/G 56.8% *vs* 69%, heterozygotes G/T 37.8% *vs* 27.9% and homozygous mutant T/T 5.3% *vs* 2.9% respectively. The increased frequency of G/T+T/T variant genotypes in AML patients was statistically significant compared to controls ($p<0.05$). Stratification of patients according to gender revealed no differences in genotypic distribution of *CYP2B6* genotype between male and female AML patients. Among females, the frequency of the mutant variant T was significantly increased in AML patients comparing to the healthy controls (homozygous mutant T/T 5.2% vs 0%, $p<0.05$). No homozygous mutant T/T genotype was observed in patients with favorable prognosis chromosome abnormalities[inv(16), t(8;21), t(15;17)]. Interestingly, patients with aberrations of chromosomes 5 and/or 7 showed a statistically increased frequency of the homozygous mutant T/T genotype. Stratification of patients according to FAB classification and age groups revealed no differences in the mutant (G/T and T/T) genotypic frequencies. **Summary and Conclusions.** The increased frequency of G/T+T/T variant genotypes in AML patients indicates a possible implication of CYP2B6 G516T polymorphism in the pathogenesis of AML. The statistically increased frequency of the homozygous mutant T/T genotype in AML patients with aberrations of chromosomes 5 and/or 7 indicates that completely lack of CYP2B6 enzymatic activity may be associated with the formation of these aberrations which confer an unfavorable prognosis in AML patients.

0640

FURTHER DELINEATION OF GENETIC LESIONS IN CORE BINDING FACTOR-ACUTE MYELOID LEUKEMIA BY ASSOCIATION OF G BANDING AND GENOMIC KARYOTYPES

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Background and Aims. Acute myeloid leukemia (AML) is a group of clonal diseases, resulting from two classes of mutation. We performed a SNP array (SNPa) in a set of core-binding factor acute myeloid leukemia (CBF-AML) patients to identify cooperating lesions, check the agreement between array and G banding analysis and detect the presence of *KIT* mutations, analyzing them in the context of arrays results**. Methods.** SNPa 6.0 experiments were carried out as recommended by the manufacturer and validated by comparative genomic hybridization array (aCGH), 180K as well as interphase FISH (iFISH) with probes for: *BCR* gene and *CBFB/MYH11* genes. Specific analysis programs were applied to each platform: Genotyping Console (SNPa) and DNA Analytics (aCGH); in addition, a thirdpart software was also used (Nexus 6.0). Analysis was performed using an effective resolution of 100Kb. Regions reported in the general population as copy number variations (CNV) were considered germline alterations and disregarded as relevant to cancer phenotype. *KIT* mutations (exons 8 and 17) were investigated by direct sequencing. Statistical comparisons by Nexus (Fisher's-exact test) were performed to determine the association between abnormalities by arrays and presence of *KIT* mutation. G-banding karyotype (KT) was performed as usual, and abnormalities described according to ISCN (2009). **Results.** Fifteen CBF-AMLs were analyzed. Many submicroscopic acquired somatic chromosomal alterations were detected by SNPa, most of them non-recurrent. Unbalanced inv(16) was detected by arrays in one case (nr 3): iFISH with CBFB/MYH11 probes confirms the presence of two breakpoint deletions. *KIT* mutations were detected in three cases. *KIT* mutation-positive cases were associated (p <0.05) either to high level gain of a 412kb region at 4q28 and a 2.2Mb segment at 16q22.1 exhibiting copy-number neutral loss of heterozygosity (CNN-LOH). Telomeric and long CNN-LOH regions (>25Mb) were detected in four out of fifteen cases. Microscopic alterations only detected by arrays were: nulisomy Y (cases 1 and 9); additional material at 1q25q44 region (case 6); interstitial 9q22 deletion (not terminal, case 13) and trisomy 8, not 9 (case 7). In one case, SNPa was not able to detect trisomy 22 in a low frequency mosaic. iFISH for 22q11.2 region (*BCR* gene) confirms this abnormality, as showed by KT. **Conclusions.** SNPa added valuable information to KT, but clinical significance of all these abnormalities still deserves validation. Analyzed cases are genomically heterogeneous by arrays. CNN-LOH regions were frequent in these CBF-AML cases. Regions found altered by arrays could be sites harboring new disease-related genes. Submicroscopical alterations in *KIT* mutation-negative cases could be the cooperating lesions required for clonal evolution.

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NEW QUANTITATIVE METHOD TO IDENTIFY NPM1 MUTATIONS IN ACUTE MYELOID LEUKAEMIA

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Background. Somatic mutations in the NPM1 gene, which encodes for nucleophosmin, have been reported to be the most frequent genetic abnormalities found in acute myeloid leukaemia (AML). Their identification and quantification remains crucial for the patients' residual disease monitoring. **Aims.** We investigated a new method that could represent a novel reliable alternative to sequencing for its identification. **Methods.** This method was based on high-resolution melting analysis in order to detect mutated patients and second, on an allele-specific-oligonucleotide real-time quantitative polymerase chain reaction (ASO-RQ-PCR) for the identification and quantification of NPM1 mutations (NPM1m) transcripts (Figure 1). Few patients carrying known NPM1m enabled us to set up a table with the different primers' ΔCT values. We then analysed a series of 337 AML patients' samples for NPM1 mutational status characterization, and confirmed the ASO-RQ-PCR results by direct sequencing. Analysis was performed by comparative cycle threshold (CT) method of relative quantification giving the amount of target, normalized to the ABL gene. A few patients (1-3 depending on mutation types) carrying known NPM1m were analysed in parallel in order to build an accurate and reliable table as follows: the ΔCT = CT(NPM1m) - CT(ABL) were calculated from each known mutated sample. **Results.** We identified NPM1 mutations in 86 samples (69 carried type A, 10 type B, 1 type C, 5 type D and 1 type P). Mutation types obtained by using ΔCT method were confirmed by direct sequencing for 36 samples (30 with mutation type A, 2 type B, 1 type C, 2 type D, and 1 type P) and none of them revealed any other mutation other than the one we identified with our ΔCT method. Two samples identified by HRM screening step showed ΔCT values which did not correspond to any of these mutation profiles and direct sequencing revealed rare type M and Q *NPM1* mutations. **Conclusions.** The results were fully correlated in 100% of the 36 sequenced samples. We also detected other rare NPM1m in two samples, that we confirmed by direct sequencing.This highly specific method provides a novel quick, useful and costless tool, easy to use in routine practice

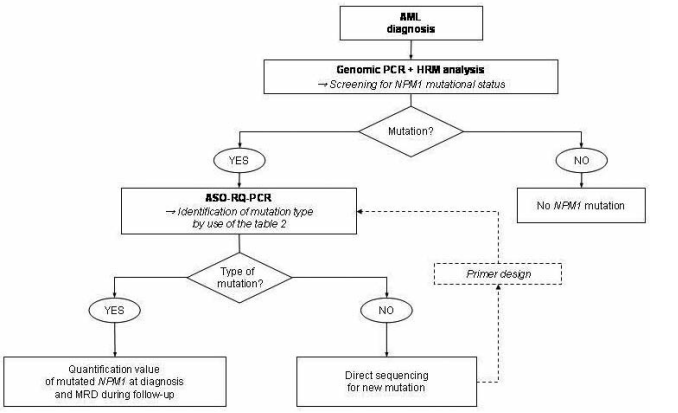

Figure 1. Strategy to identify NPM1 mutations.

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CEBPA DOUBLE-MUTATED ACUTE MYELOID LEUKEMIA DISPLAYS A DISTINCTIVE IMMUNOPHENOTYPIC PROFILE

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Background. CEBPA double-mutated (CEBPA-dm) acute myeloid leukemia (AML) has been well established as a separate entity, as stated by WHO, due to distinctive gene expression profile, microRNA profile and a relatively favorable outcome. However, no specific immunophenotypic features have been described so far in this subset. A tendency to expression of CD15 and CD7 has been reported (Lin *et al*, Clin Cancer Res 2005) but these antigens are quite commonly expressed by AML blasts. Of note, given the high variability of type and site of mutations, the identification of CEBPA-dm cases requires whole gene sequencing. CEBPA mutational analysis is thus generally focused on normal karyotype AML, in the absence of NPM1 and FLT3-ITD mutations, since they appear mutually exclusive with a CEBPA double-mutated status. **Aims.** Our aim was to analyze systematically the phenotypic profile of whole BM at AML diagnosis, in order to highlight a CEBPA-dm specific profile, that could be used as a reliable screening method for gene mutations. **Methods.** Flow cytometry: A comprehensive immunophenotypic assessment of bone marrow (BM) cells was carried out on 100 AML cases. Data acquisition was performed using FACSCalibur (Becton Dickinson); for data analysis, Infinicyt (Cytognos) software was used. Some major BM compartments (immature, neutrophil, monocytic, erythroid, basophil) were identified on the basis of FSC/SSC properties and reactivity for CD45/CD34. Overall, 82 phenotypic parameters were defined and expressed as percentage of positive cells for a given antigen within a cell compartment and/or its mean fluorescence intensity. Twenty-one normal marrows were studied by the same approach to set the reference frame. Each individual value was normalized to the mean value obtained for each parameter in normal BM and a log2 transformation was applied to normalized values. Ward's hierarchical clustering analysis of the normalized log2 ratios was performed.CEBPA mutational analysis: Total cellular DNA was extracted using the QIAmp DNA Blood Mini Kit (Qiagen, Chatsworth, CA). The entire CEBPA coding region of the gene was amplified using three overlapping PCR primer pairs as previously described (Pabst *et al*, Nat Genet 2001). PCR products were verified on agarose gel electrophoresis, and sequenced in both directions. **Results.** The results of the analysis are showed in figure 1. AML cases were categorized according to WHO; among genotypic subgroups, those with complex karyotype were gathered separately; unclassifiable cases were grouped upon morphology from M0 to M7. The analysis confirmed clustering of well-defined genotypic forms, such as t(15;17) (n=4), t(8;21) (n=5) and inv(16) (n=8). Six cases carried bi-allelic mutations of CEBPA. No single phenotypic abnormality did result specifically associated with CEBPA-dm disease group. Clustering of CEBPA-dm cases was essentially provided by the simultaneous

assessment of all phenotypic parameters within all BM cell compartments. **Summary and Conclusions.** The systematic evaluation of phenotypic parameters, routinely used in the diagnostic workout of AML, provides clustering of CEBPA-dm cases. Beyond confirming the biological homogeneity of this subset, our approach might provide a reliable and relatively straightforward tool for screening CEBPA-dm cases, thus leading to prompt availability of a crucial prognostic information since the outset.

Figure 1.

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METNASE (SETMAR) AND HPSO4 (HPRPF19) EXPRESSION IN HEMATOLOGICAL CANCERS

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Background. Balanced chromosomal translocation is a major event in both leukemia and malignant lymphomas, which characterize the molecular phenotype of the disease. The transposase domain protein Metnase promotes DNA double strand repair via non-homologous end-joining pathway and has previously been shown to suppress the development of chromosomal translocations. The human protein Pso4 (hPso4) has been shown to interact with *Metnase* thereby enhancing the DNA double strand breaks repair mechanism. **Aims.** We wished to establish a sensitive and specific qPCR assay for quantification of the expression of *Metnase* and *hPso4* with the intent to measure and compare the transcription levels of *Metnase* and *hPso4* in patients within acute myeloid leukemia (AML), chronic myeloid leukemia (CML), mantle cell lymphoma (MCL) and in a cohort of healthy individuals as control.*Methods*: Peripheral blood (PB) and bone marrow (BM) samples were collected from the time of diagnosis. Primer and probes were designed to span exon-exon junctions for mRNA specificity. All samples were normalized to the cell line Granta-519, thereby defining the *Metnase and hPso4* levels in Granta-519 as 1. *Beta-2-microglobulin* and *beta-glucuronidase* were used as reference genes. The qPCR results were analyzed using a Wilcoxon Rank system (Mann Whitney test) in order to find any differently expressed subgroups of patients. **Results.** We detected a significantly higher expression of *Metnase* in AML compared to healthy individuals. There was no difference in the expression of *Metnase* in the cohort of AML patients harboring a chromosomal translocation compared to the group of AML patients without a translocation.

Table 1. Expression of Metnase and hPso4.

	Patients groups	N	Median expression/range Normalized to Granta-519	Compared to healthy individuals p-value
Metnase	Healthy individuals, PB	19	0.127 (0.075-0.26)	-
	Healthy individuals, BM	18	0.314 (0.18-0.48)	-
	AML, translocation neg, PB	27	0.98 (0.08-11.29)	<0.0001
	AML, translocation neg, BM	21	1.26 (0.34-11.78)	<0.0001
	AML, translocation pos, PB	16	0.58 (0.14-1.29)	<0.0001
	AML, translocation pos, BM	12	0.92 (0.60-1.39)	<0.0001
	CML, PB	24	0.13 (0.04-0.73)	0.8641
	CML, BM	12	0.34 (0.11-0.96)	0.6115
	MCL, PB	19	0.31 (0.06-1.76)	0.0019
	MCL, BM	11	0.45 (0.1-0.63)	0.3687
hPso4	Healthy individuals, PB	19	0.286 (0.17-0.73)	-
	Healthy individuals, BM	18	0.396 (0.06-2.01)	-
	AML, translocation neg, PB	27	0.58 (0.08-2.17)	0.0022
	AML, translocation neg, BM	21	0.787 (0.004-3.29)	0.4639
	AML, translocation pos, PB	16	0.33 (0.21-0.89)	0.1361
	AML, translocation pos, BM	12	0.55 (0.26-0.75)	0.6597
	CML, PB	24	0.09 (0.031-0.26)	<0.0001
	CML, BM	12	0.11 (0.02-0.27)	<0.0001
	MCL, PB	19	0.55 (0.046-2.86)	0.0007
	MCL, BM	11	0.50 (0.26-2.8)	0.5896

Table 1 shows the expression levels of *Metnase* in healthy individuals, AML, CML, and MCL. The expression of *hPso4* (Table 1) was significantly decreased in CML compared to the cohort of healthy individuals. The contrary situation was found in PB MCL where both the expression levels of *Metnase* and *hPso4* were significantly increased. The expression ratio of *hPSo4*/*Metnase* was >1 in MCL and healthy individuals while the ratio of *hPSo4*/*Metnase* was <1 in the group of AML with and without translocation and CML patients.*Summary:* We designed a qPCR assay to detect the expression of *Metnase* and *hPsO4* in hematological cancers. Initially, we hypothesized that there was a higher *Metnase* expression in AML patients without translocation compared to those with a translocation, but interestingly, we found no significant difference between those groups but a significant increased expression in both AML subgroups compared to the cohort of healthy individuals. Moreover, we found a significantly reduced expression of *hPso4* and no differences of *Metnase* expression in the cohort of CML patients compared to healthy individuals. Finally, there was a significant higher expression of both *Metnase* and *hPso4* in MCL PB samples. This study elucidates the expression patterns of two of the co players in the non-homologous end-joining pathway with impact on the chromosomal events in subgroups of hematological cancers.

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CLONAL MUTATIONS IN MATURE MONOCYTIC CELLS IN ACUTE MYELOID LEUKAEMIA

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Background. In AML, genetic anomalies are sequentially acquired causing leukemogenesis. The ontogenic stage at which these changes occur and their impact on differentiation to mature myeloid cells such as monocytes, remains unclear. Leukemic mutations in monocytes, which are long-lived cells, could have implications for the interpretation of molecular minimal residual disease (MRD) monitoring. Furthermore, clonal monocytes might conceivably acquire additional leukemic genetic anomalies. **Aims.** To investigate the presence of recurrent AML-specific mutations in the mature monocytic (CD14+) component of AML with and without morphological evidence of maturation. **Methods**. The AutoMACS technique was used to separate CD14+ cells from whole blood or bone marrow in diagnostic and follow up samples from patients with AML/MDS. Purity of separation was confirmed by flow cytometry (=/>95%) and DNA was extracted from both the CD14+ fraction and the whole cell sample. Both fractions were routinely screened for mutations in *FLT3*, *NPM1, IDH1* and *IDH2.* Biallelic *CEBPA* mutations were identified in one case.

Table 1. Comparison of FLT3-itd allelic ratios in monoblastic/monocytic versus non-monocytic subtypes of AML

Non-monocytic AML (Allelic ratio whole/CD14)	Monocytic/Monoblastic AML (Allelic ratio whole/CD14)
0.70/0.05 (myelomonocytic)	0.72/0.73 (monocytic)
0.22/0.08 (with maturation)	0.61/0.90 (monocytic)
0.69/0.15 (minimal maturation)	0.63/0.67 (monocytic)
2.50/1.08 (without maturation)	0.50/0.32 (monoblastic)

Results. Cases of AML (n=29) and MDS (n=6) were analysed, with the majority of patients >60years (n=23). All were classified as per WHO criteria and AML cases morphologically grouped as follows; AML with dysplasia (n=8), AML with minimal/no maturation (n=3), AML with maturation (including core-binding factor and myelomonocytic AML) (n=9) and monoblastic/monocytic AML (n=6). *FLT3*-internal tandem duplication (itd) (n=9) and *NPM1* (n=7) mutations occurred most frequently and were predominantly seen in monoblastic/monocytic AML subtypes (56%, n=5 and 43%, n=3, respectively). *IDH1* and *IDH2* mutations were seen in 10% of AML cases. All mutations in the whole cell population were present in the CD14+ fraction. In addition, FISH studies demonstrated trisomy 8 in CD14+ cells from monoblastic AML with trisomy 8. These

findings suggest that monocytic maturation of leukaemia initiating cells can occur following the acquisition of clonal genetic aberrations in AML. Fragment analysis of DNA for *FLT3*-itd was used to calculate mutated to wild-type allelic ratios (ARs) in the whole cell and CD14+ fraction, providing a semi-quantitative measure of leukaemia burden in each. ARs in monoblastic/monocytic AML were highly comparable between the whole and CD14+fraction. In non-monocytic AML, the CD14+ AR was considerably lower than in the whole cell population, Table 1. It can be inferred that clonal monocytic maturation occurs to a greater degree in monoblastic/monocytic subtypes of AML but may also be seen in limited cell populations in AML with minimal or no evidence of maturation.Pre and post treatment CD14+ fractions were obtained in 6 cases with mutations in *FLT3, NPM1* or *CEBPA*. All mutations in the CD14+ fraction correlated with the whole cell population post treatment, suggesting that clonal mature monocytic cells show similar treatment susceptibility to the whole blast population. **Conclusions.** We have identified genetic abnormalities in CD14+ cells from various AML subtypes (excluding APML), suggesting that monocytic differentiation of the leukemic clone can occur even in non-monoblastic/monocytic AML. The pathogenic relevance of these differentiated subclones is unclear but further functional and quantitative assays will help to ascertain their significance.

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THE ROLE OF CBL IN RELATION TO RAS-PATHWAY ACTIVATION IN PEDIATRIC AML

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Background. Leukemogenesis in acute myeloid leukemia(AML) is hypothesized to evolve from mutations disrupting proliferation and differentiation. Several mutations resulting in activation of the RAS-pathway have been described in AML and *MLL*-rearranged ALL. Recently, mutations in the *Casitas B lineage lymphoma(CBL)* gene were reported to be involved in RAS-pathway activation in various myeloid malignancies. So far, the role of *CBL* in pediatric AML is unknown. **Aims.** We aimed to study the role of *CBL* mutations and CBL expression in pediatric AML and *MLL*-rearranged ALL. **Methods.** We performed mutation analysis in 277 initial and 33 relapse pediatric AML samples, and 18 *MLL*-rearranged infant acute lymphoblastic leukemia(ALL) samples. To further evaluate the functional role of CBL in pediatric AML, and RAS-pathway activation, we studied *CBL* mRNA and protein expression and performed *CBL* RNA interference knock-down experiments. **Results.** We identified only two mutant cases(0.7%) in newly diagnosed pediatric AML, and none in relapsed cases. One mutant case was *MLL*-rearranged; the other had a normal karyotype. No *CBL* mutations were present in infant ALL cases. *CBL* mRNA expression in mutated cases did not differ from non-mutated AML cases nor between any of the cytogenetic groups. Western blotting of patient samples showed no significant difference in protein expression of the two *CBL* mutants versus nine non-mutant AML cases(p=0.8)., and protein expression had a poor correlation with mutation status or *CBL* mRNA expression as determined by gene expression profiling and confirmed by RT-qPCR(Spearman r=0.17). Also, phosphorylated extracellular signal-regulated kinase(pERK) and CBL protein expression did not correlate. Nevertheless, *CBL* RNA interference knock-down experiments resulted in an upregulation of pERK protein expression in the Kasumi-1 cell line, with a maximum of 3-fold at t=48 hours. **Summary and Conclusions.** In conclusion, we report a very low *CBL* mutation frequency(0.7%) in pediatric AML and *MLL*-rearranged infant ALL and poor correlation with expression of *CBL* mRNA, CBL protein and pERK protein. However, enforced decreased CBL protein expression led to RAS-pathway activation in a pediatric AML cell line.

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THE PHARMACOKINETICS OF ELACYTARABINE IN AML PATIENTS

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Background. Elacytarabine is an elaidic acid ester derivative of cytarabine. Like cytarabine, elacytarabine is metabolized to the active ara-CTP and to ara-U. Elacytarabine has reached phase III of clinical development for treatment of relapsed or refractory Acute Myeloid Leukaemia (AML). The current study is using the same dose, schedule and formulation as in the ongoing pivotal CLAVELA study. **Aims.** To characterize the pharmacokinetics (PK) of elacytarabine and its metabolites ara-C and ara-U after a 120-h continuous i.v. infusion (CIV) of the recommended dose of elacytarabine to patients with relapsed or refractory AML. The study is also assessing the efficacy, safety and tolerability of elacytarabine in this patient population. **Methods.** Elacytarabine for infusion 7.5 mg/mL was administered at 2000 mg/m^2/d as a CIV for 120-h to adult patients with relapsed or refractory AML. Blood samples for PK analysis were collected at 12 predefined time points prior to, during and after administration. The plasma concentrations of elacytarabine, ara-C and ara-U were determined by a validated LC-MS/MS method. PK parameters like C_{max}, T_{max}, AUC, CL, V_{ss} and $T_{1/2}$ were calculated using both compartmental and non-compartmental analyses. **Results.** Thirteen patients [10 males, 3 females, median age 63 years (range 48-77), ECOG PS 0-1] were treated with elacytarabine. All patients had previously been treated with cytarabine, and received elacytarabine as either first or second salvage treatment.Elacytarabine and ara-C were detected in plasma up to 24 h after end of infusion. The plasma concentrations of elacytarabine and ara-C declined rapidly once the infusion was stopped. Using non-compartmental analysis, the median half-life and CL for elacytarabine were 9.9 h and 4.31 L/h/m^2. For ara-C and ara-U the half-lives were 3.2 and 9.7 h, respectively.Five patients achieved a CR/CRi and one patient had partial remission. The most frequently reported related non-hematologic adverse events (CTCAE grade ≥ 3) were febrile neutropenia, infections, hypokalemia and increased cholesterol. **Summary and Conclusions.** Elacytarabine is a novel anti-leukemic agent for treatment of patients with advanced AML. The PK of the drug and its metabolites as well as its toxicity and efficacy were characterized after CIV for 120 h in this patient population and showed that the drug has features making it a promising therapeutic for this disease.

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OUTCOME OF HIGH-RISK AND REFRACTORY AML/MDS PATIENTS AFTER FLAMSA SEQUENTIAL CHEMOTHERAPY REGIMEN FOLLOWED BY REDUCED-INTENSITY CONDITIONING AND ALLOGENEIC HEMATOPOEITIC STEM CELL TRANSPLANTATION

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Aims. This retrospective analysis aimed to assess the outcome of 40 patients with refractory or high risk AML/MDS who received FLAMSA sequential chemotherapy. **Methods.** There were 30 males and 10 females with a median age of 52 years (32-66). Diseases characteristics were: progressive or refractory disease after rescue treatment for first relapse (n=21), early relapse without any further salvage therapy (n=4), and primary induction failure (n=4). The series also included 7 patients with high risk MDS and 4 patients in first CR but having a very poor prognosis. The FLAMSA regimen included Fludarabine (30 mg/m²/d), cytarabine (2 g/m²/d) and amsacrine (100mg/m²/d) from day -12 to day -9. After 3 days of rest, a RIC regimen was administered. In 28 patients, the RIC regimen included 4 Gy. TBI, ATG 5mg/kg total dose, and cyclophosphamide 40mg/kg in case of matched related donors, and 60 mg/kg for unrelated or mismatched donors. In the remaining 12 patients, TBI was replaced by I.V. Busulfan 3.2 mg/kg/d for 4 days. Eighteen patients were transplanted using an HLA identical sibling donor, and 22 received transplant from an unrelated donor. **Results.** After allo-HSCT, 39 patients (97.5%) engrafted. In the CR

group (n=4), after a median follow-up of 5 months (range, 3-31) all patients were still alive in CR at last follow-up. In the remaining 36 patients, 9 patients developed acute GVHD ≥2 with a cumulative incidenceat 3months of 18% (95%CI, 10-26). At day 90 post HSCT, 23 (64%) patients achieved hematological CR, and 14 of the 23 remained in CR at last follow-up. After a median follow-up of 6 months (range, 1-60), the 2-years probability of OS was 30% (95%CI, 17-52), and the 2-years probability of PFS was 29% (95%CI, 17-50). The cumulative incidence of relapseat 1year was 25% (95%CI, 18-33). Interestingly, none of the patients who received Busulfan instead of TBI, relapsed. The cumulative incidence of TRMat 3months and 1 year were 14% (95%CI, 8-20) and 22% (95%CI, 15-29), respectively.In the multivariate analysis there was a significantly worsened PFS in patients who received transplant from a mismatched donor (HR=3.6; [95%CI, 1.3-10] p=0.01). Also, when considering disease relapse, there was a highly significant impact of the type of RIC regimen (in favour of a FLAMSA regimen without TBI (HR=0; [95%CI, 0-0] p<0.0001). **Conclusions.** A modified FLAMSA regimen incorporating I.V. Busulfan instead of TBI is likely to allow better long-term disease control, warranting prospective evaluation.

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TREATMENT OUTCOME OF BRAZILIAN PATIENTS WITH ACUTE MYELOID LEUKEMIA: AN ANALYSIS BASED ON THE EUROPEAN LEUKEMIANET CLASSIFICATION SYSTEM

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Background. It has been increasingly acknowledged that assumptions drawn from classical clinical trials are not always trustworthy clues for therapy decision-making. In particular in developing countries, where the result of the treatment of hematologic malignancies is poor, it is unknown whether European LeukemiaNet (ELN) recommendations for Acute Myeloid Leukemia (AML) risk stratification would be applicable in a real-life setting. **Aims.** To assess how to improve the risk stratification of Brazilian patients with AML, taking better advantage of the new ELN recommendations in addition to well recognized prognostic markers. **Methods.** Two hundred and seventeen consecutive patients treated at two University Hospitals between 2001-2010 with age between 15 and 65 years were studied. Age, gender, WBC count, karyotype, *NPM1*, FLT3-ITD, *CEBPA* and MLL-PTD mutations, and the *BAALC* gene expression levels were analyzed. Patients were treated with conventional chemotherapy: daunorubicin (60 mg/m^2/d for 3 days) and cytarabin (200 mg/m^2/d for 7 days) as induction, followed by two or three courses of consolidation therapy with high-dose cytarabin (above 1 g/m^2/d). Median follow-up time for surviving patients was 22 months. The median age was 42 years and 98 (45%) were male. Patients were assigned to the genetic groups proposed by the ELN as follows: favorable (n=83), intermediate I (n=61), intermediate II (31) and adverse (n=20). Direct sequencing of PCR products was performed to detect *NPM1* mutations (*NPM1*mut). *CEBPA* mutations were identified by DHPLC or direct sequencing. FLT3-ITD detection was performed by PCR. MLL-PTD was detected by RQ-PCR. The relative expression levels of *BAALC* gene were measured by RQ-PCR, and patients were dichotomized at *BAALC*'s median expression into low and high expressers. **Results.** The estimated five-year overall survival (OS) for all patients was 19%. Univariate analyses determined that age, genetic risk group, FLT3-ITD, MLL-PTD and WBC count were significant prognostic factors. Patients older than 45 years presented shorter OS in comparison to those ≤ 45 years (P=0.008). The mean OS for favorable, intermediate I, intermediate II, and adverse groups were 43, 13, 16 and 13 months, respectively (P=0.03), while the disease-free survival (DFS) was 61, 34, 42 and 5 months, correspondingly (P=0.001). Patients harboring either FLT3-ITD or MLL-PTD presented shorter OS in comparison to patients with wild-type genes (P=0.03 and P=0.002, respectively). WBC count above 50x10^9/L also predicted shorter OS (P=0.01). *CEBPA* mutations were associated to complete remission (CR) achievement (P=0.01) and longer OS (P=0.08). Multivariate analysis detected age, MLL-PTD and WBC count as independent prognostic factors for OS. Further stratification of intermediate risk group identified patients with distinguishable outcomes. The DFS was favorably impacted by *NPM1*mut (P=0.07) and *CEBPA* biallelic mutations (*CEBPA*bm) (P=0.08), or the absence of FLT3-ITD (P=0.06). *CEBPA*bm were also associated to longer OS (P=0.01), and *NPM1*mut to CR achievement (P=0.01). **Conclusions.** Brazilian patients indeed presented poorer outcome than those enrolled in the US and Europe clinical trials. Patients classified into intermediate I, intermediate II, and adverse groups presented similar outcome. The assessment of additional prognostic factors would help to guide patients' management when ELN recommendations fail to translate into clinical outcome.

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PROGNOSTIC IMPACT OF MONOSOMAL KARYOTYPE IN ADULT ACUTE MYELOID LEUKEMIA

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Background. Acute Myeloid Leukemia (AML) is a heterogeneous entity and comprises a group of hematologic neoplasms with distinct clinical and genetic features. Age and karyotype at diagnosis remain the most important independent prognostic factors in adults with AML. Recently, a new cytogenetic category was introduced, Monosomal Karyotype (MK), defined by the presence of 2 or more monosomies, or a single monosomy in the presence of structural abnormalities. This category has been described as having an adverse prognosis and as adding prognostic information even in patients with complex karyotype (CK). **Aims.** To evaluate the prognostic impact of MK in adults diagnosed with AML. **Methods.** From January 1998 to December 2010, 313 patients were diagnosed with AML at our Institution. For the purpose of this study, we excluded patients with Acute Promyelocytic Leukemia (45 patients) and patients with unknown karyotype at diagnosis (55 patients; not requested or not performed due to insufficient metaphasis). The remaining patients were divided according to karyotype into 3 prognostic groups: favorable (16.0%), intermediate (51.6%) and adverse (32.4%). Considering only the patients from the adverse prognostic group, 26 patients fulfilled criteria for MK and therefore this group was further divided into MK(+) and MK(-). Statistical analysis was performed with SPSS®Statistics v19. **Results.** Comparing both groups, MK(+) and MK(-), patients with monossomal karyotype were slightly older (median age of 64 years vs. 56 years, p=0.059), were predominantly of the female gender (75.0% vs. 32.6%, p=0.003) and presented a more elevated peripheral blast count (40.0% vs. 9.0%, p=0.065). No other differences were found regarding presentation symptoms, performance status, leukocyte count, marrow blast count or LDH at diagnosis. Induction therapy was equally distributed between both groups.Complete remission rate (CR) was similar between the groups analyzed (55% in MK(+) vs. 63.2% in MK(-), p=0.343). The incidence of disease recurrence was slightly more elevated in the MK(+) group (63.6% vs. 50%, p=0.352) with a median time to relapse of 3.2 months in MK(+) and 8.4 months in MK(-), p=0.196.MK(+) group had a worse overall survival (OS, median of 2.1 months vs. 10.0 months, p= 0.002; Figure 1a) and a worse disease-free survival (DFS, 4.0 months vs. 11.5 months, p=0.032; Figure 1b).On multivariate analysis of pretreatment characteristics only age (p=0.014) and presence of MK (p=0.004) were identified as independent prognostic factors.Analyzing further the patients with CK, we divided them in 2 groups: patients with both CK and MK (CK+MK+, 20 patients) and patients with CK without MK criteria (CK+MK-, 18 patients). No differences were found between the 2 groups in terms of CR and relapse rate. However, patients with CK+MK+ had a worse OS (median OS of 2.4 months vs. 10.0 months, p=0.008) and a worse DFS (median DFS of 3.2 months vs. 13.2 months, p=0.006). **Conclusions.** Our study reveals that the presence of MK is associated with an adverse outcome in AML and also suggests that the worse prognosis associated with the presence of CK might be related to the inclusion of MK patients in that group.

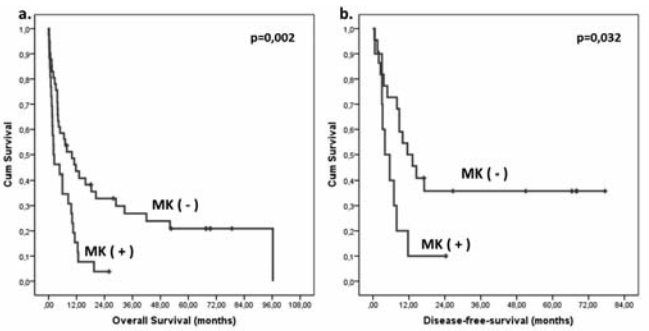

Figure 1. Overall survival (a) and disease free survival (b) of MK$^+$ and MK$^-$patients.

0650

REDUCING DOSAGE OF IDARUBICIN AND FLAG FOR PATIENTS YOUNGER THAN 65 YEARS WITH RESISTANT ACUTE MYELOID LEUKEMIA: A PROSPECTIVE, MULTICENTER PHASE II STUDY

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Background and Methods. We previously assessed continuous infusion (CI) of fludarabine and cytarabine plus idarubicin (CI-FLAG ver. 1) for patients under 65-years old with resistant acute myeloid leukemia (AML). Induction chemotherapy consisted of idarubicin (IDA,12 mg/m^2 on Days 1-3), plus fludarabine (FLU, 30 mg/m^2/day) and cytarabine (ARAC, 1,000 mg/m^2/day) on Days 1-5 as a 24-hr CI. G-CSF was added on Days 1-5. In response to induction, 31.6% patients achieved CR and 68.4% failed treatment; of the latter, 42.1% had aplasia. We concluded that although CI-FLAG ver. 1 was effective for eradicating blasts, it carried a high risk of toxicity and reduced doses were recommended for CI-FLAG. Therefore we revised the protocol (CI-FLAG ver. 2) by reducing the dose of IDA (6 mg/m^2/d for 3 days) and ARAC (1000 mg/m^2/d on days 1 through 2, and then 200 mg/m^2/d on day 3 through 5 as CI). The schedule and dose of FLU and G-CSF were same as the original protocol. Here we present the result of CI-FLAG ver. 2 and the comparison with CI-FLAG ver. 1. **Results.** Total 39 patients were enrolled in CI-FLAG ver. 2. Patients were of median age 42 years (range, 16-62 years); of these, primary refractory disease in 26 (66.7%), early relapse in 12 (30.8%), and relapse after hematopoietic cell transplantation (HCT) in 1 (2.6%). Risk groups were favorable in 17.9%, intermediate in 61.5% and poor in 15.4%. Overall CR rate was 41%. CR, CRi, CRp and PR were achieved in 33.3%, 5.1%, 2.6% and 7.7% patients, respectively. A median day to CR was 36 (24-71). The main reasons of treatment failure were resistance in 12 (30.8%), intermediate course in 4 (10.3%), morphologic relapse in 2 (5.1%) and aplasia in 2 (5.1%). Three patients tried re-induction but all failed achieving CR. 25.6% patients received salvage chemotherapy after failure of CI-FLAG ver. 2. Twenty seven patients received SCT (13 after remission, 7 after salvage chemotherapy, 6 as salvage HCT). When comparing outcomes between ver. 1 and ver. 2, there were no difference in terms of CR rate (18.4 vs. 33.3%, p=0.136), all CR rate (31.6 vs. 41%, p=0.389) and objective response rate (ORR, 31.6 vs. 46.2%, p=0.190). Cumulative incidence of relapse among patients who had achieved CR were not different (37.7 vs. 63.2%, p=0.277). The median overall survivals showed only the trend of longer survival in ver. 2 (2.47; 95% CI, 0.073-4.861 vs. 6.32; 95% CI, 2.452-10.180 months; p=0.103; Figure 1). Among intermediate risk patients, there were significantly high response rate favoring ver. 2 in terms of CR rate (6.7 vs. 41.7%, p=0.028), all CR rate (20 vs. 50%, p=0.061), ORR (20 vs. 54.2%, p=0.035) and overall survival (2.6 vs. 7.2 months, p=0.093). **Conclusions.** Our prospective study for highly refractory AML showed substantially improved rate of response compared with our previous study. However there was high rate of resistance by reducing dosage at expense of decreasing aplasia. The reduced dose of CI-FLAG might be most beneficial for intermediate risk group.

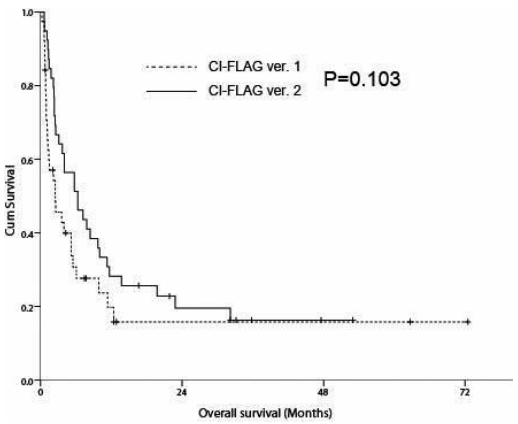

Figure 1. Overall survival.

0651

CLINICAL AND LABORATORY CHARACTERISTICS OF ACUTE MYELOID LEUKEMIA PATIENTS WITH PARTIAL TANDEM DUPLICATION OF THE MLL GENE

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Background. Partial tandem duplications of the MLL gene (PTD-MLL) are one of common molecular abnormalities in acute myeloid leukemia (AML) and its detection is considered as a marker of unfavorable prognosis. **Aims.** To establish the incidence of MLL-PTD-positive [MLL-PTD(+)] AML in Bulgarian population and to search for an association with biological, clinical, morphological, immunophenotypic, and molecular characteristics of patients **Patients and Methods.** Peripheral blood and/or bone marrow samples of 246 (120 female; 146 males) adult AML patients, with mean age of 54.7±16.8 years were studied. Diagnosis and classification of AML were established using the standard criteria by means of routine hematological tests, morphology, cytochemistry, immunophenotyping by flow cytometry, conventional cytogenetics and reverse transcription polymerase chain reaction (RT-PCR). MLL-PTD status was determined using RT-PCR. **Results.** In total, MLL-PTD mRNA was found in 14 AML patients (5.7%). The MLL-PTD(+) group was characterized by a distinct predominance of male compared to female gender (11/126; 8.7% vs. 3/120; 2.5%; p=0.051); a clear tendency for higher frequency of therapy-related AML compared to *de novo* AML [4/27 (14.8%) vs. 10/219 (4.6%); p=0.054], and absence of fusion transcripts associated with recurrent genetic abnormalities, such as BCR-ABL; AML1-ETO; CBFb-MYH11; PML-RARA; MLL-AF6; MLL-AF9; and DEC-CAN. The remaining characteristics were heterogeneous and unspecific: the median age was 58.5 years (range 24 - 83 years); the median white blood cells count - 23.0x10^9/l (range 1.2x10^9/l -130x10^9/l); median platelets count - 41.1x10^9/l (range 19x10^9/l - 336x109/l), median hemoglobin concentration - 71.5 g/L (range 45 g/L - 105 g/L) and median bone marrow blasts/blast equivalents - 57.5% (range 31% - 98%). Among *de novo* AML cases, 2 were classified as AML with myelodysplasia-related changes, and 8 as AML, not otherwise specified, including: AML without maturation (n=1); AML with maturation (n=1); acute myelomonocytic leukemia (n=4); acute monoblastic and monocytic leukemia (n=1) and acute erythroid leukemia (n=1). By flow cytometry, 8 patients were CD34 and/or CD117-positive, and co-expression of lymphoid markers was seen in 9 (64.3%) cases, however, no one fulfilled the criteria for biphenotypic leukemia. Co-expression of FLT-ITD was detected in 3 cases (21.4%). Complete remission was achieved in only two cases (20.0% of patients in whom standard therapy was applied), and the median overall survival was 5 months. **Conclusions.** The estimated prevalence of MLL-PTD in our study does not differ from the generally reported values. As a whole, the main clinical and laboratory characteristics of MLL-PTD(+) varied in a significant degree, however poor response to therapy seems to be a common feature. **Acknowledgements:** This work was supported by the National Science Found grant No. CVP/01-0119 - DO-02-35/09

0652

° POSTMORTEM EPIDEMIOLOGY OF INVASIVE FUNGAL INFECTION (IFI) IN HEMATOLOGIC MALIGNANCIES: A STUDY OVER A 10 -YEAR PERIOD (2002-2011) AT A HEMATOLOGIC TERTIARY CARE DEPARTMENT

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Background. The lack of sensitive and specific diagnostic tools make difficult the IFI diagnosis in hematologic malignancies. Recent several reports outlined the increased incidence of moulds and yeast, which are difficult to identify early. Postmortem data may be useful: a) to monitor the frequency and the disease patterns , b) to better understand local epidemiology , c) to improve antifungal treatment strategy. **Aims**.The aim of this retrospective review is to show the local epidemiology and the prevalence at autopsy of proven IFI occurring in hematologic malignancies ,at a single center over a ten -year period. **Methods.** We retrospectively evaluated 148 patients- median age 64 y, range 22-81 - with hematologic malignancies, who underwent autopsy between 2002 - 2011. Of these pts, 69 were Acute Myeloid Leukemia (AML), 11 Acute Lymphoid Leukemia (ALL), 53 Lymphoprolipherative Disorders (LPD) ,and 9 other disorders. Acute leukemia pts received systemic antifungal prophylaxis ,whereas the others pts not absorbable prophylaxis. None patient underwent transplant . An experienced pathologist

evaluated the major organ involvement and the IFI pathologic patterns . Fisher's Exact test was used to recognize either the IFI prevalence ,the main occurring pathogen and the more involved site ; a p- value of < 0.05 was considered statistically significant. **Results.** The analysis of 148 consecutive autopsies identified 34 (23 %) pts resulting to have IFI; of these, 18 (12%) were AML, 7 (4 %) ALL, 8 (5%) LPD ,and 1 other. Aspergillus spp infection occurred in 17 (50%) cases , Mucor spp in 7 (21%), and Candida spp in the remaining 10 (29 %). In acute leukemia pts invasive mould infections were prevalent, and Aspergillus spp. proved to be the leading pathogen with respect to Candida and Mucor spp. (p 0.0091), with a statistically significant prevalence in ALL (p 0.0140). In all cases, lung resulted the site more significant involved (p 0.0011). Whereas the standardized EORTC/MSG criteria applied *in vita* were conclusive for IFI in 5 (14%) pts only ,postmortem findings revealed fungal infection in 29 (86%) pts. **Conclusions.** This analysis confirms that in hematologic malignancies the IFI diagnosis *in vita* is a still unresolved issue. Despite a larger availability of antifungal treatment , acute leukemias remain the subset with higher prevalence of mould infections. Like other larger previous studies ,also in our experience, Aspergillus Spp and lung proved to be the most recurrent pathogen and site involved. The epidemiology pattern is complex and present diagnostic methods are not still completely able to identify the underlying IFI, thus autopsy rate should be increased to achieve a better knowledge of epidemiology and to critically review previous misdiagnosis.

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HIGH CURE RATES CAN BE EXPECTED IN PATIENTS WITH THERAPY-RELATED ACUTE PROMYELOCYTIC LEUKEMIA (T-APL), BUT VIGILANCE FOR THERAPY-RELATED MYELODYSPLASTIC SYNDROME OR ACUTE MYELOID LEUKEMIA IS WARRANTED

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Background. An increasing number of patients with therapy-related acute promyelocytic leukemia (t-APL) has been reported. However, little is known about long-term outcomes and treatment-related complications of these patients as many APL clinical trials restrict eligibility to those patients with *de novo* disease. **Aims.** We conducted a single institution retrospective survey to examine the early death rate, long-term outcomes, and the rate of secondary malignancies in patients with t-APL. **Methods.** APL cases occurring after chemotherapy and/or radiotherapy for a previous malignancy were defined as t-APL. All t-APL patients, diagnosed between 1992 and 2007 at Memorial Sloan-Kettering Cancer Center, were identified. Analysis included primary malignancy, prior chemotherapy, interval from diagnosis of primary malignancy to t-APL, treatment-related complications and long-term outcomes. **Results.** A total of 122 patients with APL were identified; of these, 11 patients (9.0%) were classified as t-APL. Compared to patients with *de novo* APL, patients with t-APL were older (median age 60 vs. 46 years; p=0.012) and were women (9/11 vs. 53/111 patients). The primary malignancies were breast (n=4), cervical (n=2), colorectal (n=2), ovarian (n=1), endometrial cancer (n=1), Hodgkin's (n=1) and non-Hodgkin's lymphoma (n=1). Therapy for primary malignancies included radiation alone in 3 patients, chemotherapy alone in 7 patients, and both in 1 patient. The median interval from the diagnosis of primary malignancy to the diagnosis of t-APL was 28 months (range, 10-120 months). All patients received induction therapy with ATRA +/- anthracyclines. Ten patients (90.9%) achieved complete remission, and one patient with metastatic ovarian cancer died within 7 days of starting ATRA due to multi-organ failure from her primary malignancy. At a median follow-up of 7 years (range, 5 - 14 years), 7 of the 11 patients (64%) remain alive in continuous remission; 2 patients died from the primary malignancy; and the other 2 patients developed therapy-related MDS/AML (t-MDS/AML) and died at 51 and 71 months from their initial diagnosis of t-APL, and 3 and 11 months from the diagnosis of t-MDS/AML, respectively. t-MDS/AML was observed in one out of 111 (0.9%) *de novo* APL patients. **Summary and Conclusions.** High cure rates can be expected in patients with t-APL with no increase in the early death rate. However, there appears to be an additional risk of t-MDS/AML in patients following treatment for t-APL. Although the small number of patients precludes assessment of risk factors for t-MDS/AML, the risk appears higher than 1.1% reported in patients with *de novo* APL in large clinical trials, and warrants further studies in a larger patient cohort. Furthermore, molecular studies in the future may identify unique genetic abnormalities, which may distinguish t-APL from *de novo* APL and predict for those patients destined to develop t-MDS/AML.

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PREDICTORS OF MORTALITY DURING INDUCTION TREATMENT IN APL PATIENTS TREATED WITH AIDA BASED REGIMEN

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Background. Early death during APL induction treatment remains a major concern in this highly curable disease. We aimed at identifying factors that could predict early mortality in 51 APL patients treated with the Spanish PETHEMA LPA99 trial. **Results.** Fifty one consecutive genetically confirmed APL (with t (15, 17) and /or PML-RARA) were treated in a single center (University Hospital of Tunis) between 2004 and 2010. Median age was 30 years (range 4-71). M/F was 0.64. Two (4%), 31(61%) and 18 (35%) patients were Sanz low, intermediate and high risk respectively. Median time between first symptom and diagnosis was 15 days (range 2-90).Complete remission (CR) was achieved in 43 (86%) of 50 evaluable patients (one patient died from severe bleeding before treatment onset). Eight (16%) patients developed severe bleeding and 16 (32%) differentiation syndrome (DS), severe in 11. Seven patients (14%) had early death (ED): 4 from DS and 3 from CNS bleeding. Median time from ATRA onset to ED was 8 days (range 2-22). In patients with ED and CR, respectively, median age was 40 years (range 15-71) and 30 years (range 4-64) (P=0.251) , 6/7(85%) and 24/43(55%) patients were females (P=0.134) ,71.5% and 28% were Sanz high risk (P=0.024), median time from first symptom to diagnosis was 11 days (range 3-30) and 15 days (range 2-90) (P=0.4), median baseline WBC was $17.9x10^9$/l and $3.6x10^9$/L (P=0.11) and renal failure was observed in 5 (71.5%) and 12 (27.9%) (P=0.024). By multivariate analysis, female gender (P=0.045), renal failure (P=0.021) and high WBC $>10^9$/l (P=0.041) were independent predictive factors of ED. Baseline WBC > 30 $x10^9$/l were associated with increased risk of ED by bleeding (P=0.049) and Body Mass Index (BMI) > 35 kg/m^2 of ED by DS (P=0.05).With a median follow up of 50 months, 4 year EFS and OS were 74 and 78% respectively. **Conclusions.** Female gender, renal failure and high WBC were associated with an increased rate of early death during induction therapy of APL. Despite a delay in hospital admission in many cases, interval to onset of treatment had no significant impact on ED. However, this delay may have contributed to increase WBC and impaired renal function, which in turn contributed to ED.

0655

TRANSFUSION INDEPENDENCE AND SURVIVAL IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA TREATED WITH 5-AZACYTIDINE

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Allogeneic stem cell transplantation is the only curative option for high-risk AML and MDS patients. Practically, a minority will reach such treatment. Few studies have addressed the difficult yet pertinent issue of alternative therapies for older patients deemed unfit for standard chemotherapies. Preliminary data suggest that AZA might be effective in the subgroup of advanced MDS and AML patients, resulting in 35 to 48% hematologic improvement (HI) or better response and prolonged overall survival (Silvermann 2006). In high risk MDS patients, AZA could prolong survival and delay AML transformation compared to conventional care regimen (CCR: best supportive care (BSC), low dose cytarabine or intensive chemotherapy) (Fenaux 2009). AZA therapy also increased the rate of, and prolonged, transfusion-independency (Fenaux 2010).We identified 52 consecutive AML patients (median age 68 [25-86]), treated with 5-azacytine (AZA) between January 1st 2007 and December 31st 2011 at our institution. They were not suitable for intensive chemotherapy because of high age (n=33) or comorbidities (n=9) or were refractory to initial intensive chemotherapy (n=10). AZA was administered at 100mg/m^2 sc d1-5, over 28-day cycles. Transfusion-independent (TI) patients were defined as 8 weeks without red blood cell (RBC) respectively platelet (PLT) transfusion.Our collective included 83% patients presenting with marrow blasts over 30%, thus clearly different from AZA-001 high risk MDS and RAEB-t subgroup study (Seymour 2010). At baseline, 15% (n=9) of our collective were RBC-TI, whereas 85% (43) were transfusion-dependent (TD). A similar proportion was PLT-TI, 25% (n=13) versus 75% (n=39) PLT-TD. Median overall survival of the 52 enrolled patients was 8.6 months, and twelve-month survival rate was 28% [95CI 15-43%].To evaluate TI acquisition, we selected 38 patients having received more than 2 cycles of AZA in order to achieve a minimum observation period of 8 weeks implied by TI definition. Half of RBC-TD patients (50%; 16) acquired TI on AZA treatment. Of the 28 PLT-TD patients at baseline, 50% (14) became TI. Overall, 50% (19) of the patients remained or turned out to be

both RBC and PLT-TI and 21% (8) RBC or PLT-TI.Interestingly, RBC-TI patients under therapy (16) or at baseline (6) had prolonged survival compared to RBC-TD patients (median OS 11.1 vs 5.0 months, 12-month OS 40% [95CI 19-60%] vs 13% [95CI 2-32%], P=0.0006). No difference in OS was observed whether TI was present at baseline or acquired under treatment (median OS 10.7 vs. 11.9, 12-month OS 40% [95CI 17-63%] vs. 45% [95CI 5-75%], P=NS). In multivariate analysis, baseline or acquired RBC-TI was the only significant parameter predicting survival (HR 0.36 [95CI 0.16-0.77], P=0.009) (Figure 1).We report for the first time that, despite not being curative for AML, AZA can induce transfusion independency in 50% of previously TD patients. Transfusion independence correlates to increased overall survival, regardless if present at baseline or acquired under AZA. These results suggest that transfusion independence is a strong prognostic factor in AML patients undergoing AZA therapy. This may allow extending previous observations made with MDS-WPSS risk score (Itzykson 2011), to acute leukemia.

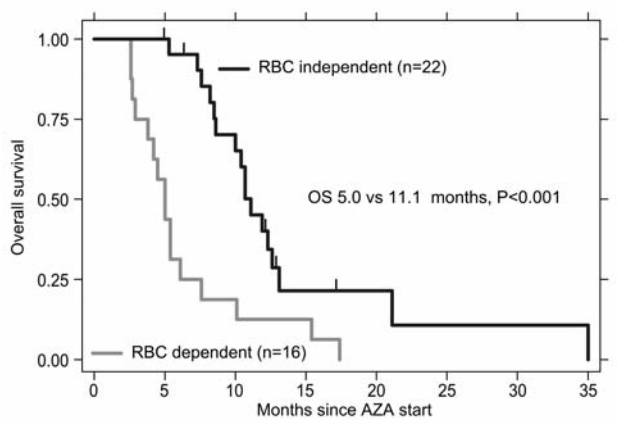

Figure 1. Survival according to RBC-transfusion independency.

0656

CONSECUTIVE INTENSIFICATION OF ACUTE LEUKEMIA TREATMENT DURING PREGNANCY - TWO DECADES STUDY

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Background. Acute leukemia (AL) in pregnant women is a rare event and it's just impossible to conduct any large prospective study. **Aims.** Since 1990NationalResearchCenter for Hematology (NRCH) has been developing the optimal treatment approaches aiming to save two lives - both a mother and a child. **Methods.** From 1990 to 2012 yy 34 pregnant women with *de novo* and 3 with relapsed AL were treated in the NRCH. The median age was 26 (19-35 years). AML was diagnosed in 16 (43,25%), APL - in 5 (13,5%) and ALL - in 16 (43,25%). In the 1st trimester the abortion was performed before the treatment (1st group n=6). In the II/III trimesters (<36 weeks) the chemotherapy was started (2nd group n=24), and delivery was planned at 36-37 weeks of gestation. If AL was diagnosed at > 36 weeks, women were delivered before the chemotherapy (3rd group n=7). At the beginning of our study in AML 7+3 was applied with 135 mg/m^2 of daunorubicin (Dauno) (n=4), than - 36 mg/m^2 of idarubicin (IDA) (n=5), and later - 180 mg/m^2 of Dauno (n=4). APL was treated with either 7+3 (Dauno 60 mg/m^2) or AIDA protocol. ALL pts received 8 weeks induction, consolidation, reinduction and 2 years maintenance. **Results.** In the 1st group the interval between abortion at 7 weeks (3-14 w) of pregnancy and treatment constituted 3 days (1-5 d); in the 3rd group between the delivery at 38 weeks (36-39 w) and treatment - 7 days (1-35).24 (AML-13, APL-3, ALL-8) underwent chemotherapy during pregnancy at 24 weeks (14-34 w). 1 ALL pt is undergoing induction chemotherapy at time of analysis. So in 10 of 23 (43,5%) CR was achieved before the delivery; 13/23 pts (56,5%) delivered without achieving CR. It's worth to note that there were no CRs in AML pts after the 1st 7+3 with 135 mg/m^2 of Dauno, so for 10 years we use IDA and Dauno 180 mg/m^2.Totally CR rate in AML and APL pts was high - 81,3% and 80%, with ED 6,2% and 20%, resistant leukemia - 12,5% and 0%, correspondingly. On the contrary CR in ALL pts was achieved only in 60%, due high resistance 20% and induction mortality 20%. It possibly is attributed to prolonged steroids (5 weeks). 12/37 pts (32,4%) are alive (2 weeks - 13 years). Antenatal fetal mortality was registered in2 pts.12 ptsdelivered by their own and17 pts- by Cesar section. One child (4%) died due to pneumonia at the 1st week of life. Other children are alive (6 mo-22 y) and healthy. It's a curious fact but in AML the majority of newborns were boys (83%) and in ALL - girls (63%) (p=0,01). **Conclusions.** Our data demonstrate that chemotherapy in pregnant women with AL should be intensive in order to achieve CR after the 1st course; induction results in AML are better than in ALL; long-term results are comparable with usualAL outcome.

0657

TREATMENT FOR ELDERLY PAIENTS WITH ACUTE MYELOID LEUKEMIA WITH AZACITIDINE RESULTS IN FEWER HOSPITALIZATION DAYS AND INFECTIVE COMPLICATIONS BUT SIMILAR SURVIVAL COMPARED WITH INTENSIVE CHEMOTHERAPY

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Background. Intensive chemotherapy administered to elderly patients with acute myeloid leukemia (AML) is associated with high treatment related complications, mortality rates and low rates of long term survival. Azacitidine has been shown to prolong overall survival compared with best supportive care in elderly AML patients with low blast counts. **Aims.** Our study looks at the differences between the mortality and morbidity outcomes of elderly AML patients who received intensive chemotherapy, azacitidine based therapy or best supportive care. **Methods.** Patients 60 years or older diagnosed with AML between January 2009 and June 2011 were included in our retrospective review. Those who passed away within less than 2 weeks of diagnosis were excluded.Intensive chemotherapy regimen comprised intravenous idarubicin 12mg/m2 for 3 days and cytarabine 100mg/m2 infusion for 7 days as induction followed by consolidation with intraveous cytarabine 1.5 gram/m2 twice daily on days 1,3 and5. Azacitidine based regimen comprised subcutaneous azacitidine 75mg/m2 daily for 7 days with or without oral valproic acid and/or all-trans-retinoic acid. Best supportive care consisted of transfusion support and antibiotic therapy for infections. Overall survival (OS) curves were estimated by the Kaplan-Meier Method, differences between other patient characteristics were analyzed with the ANOVA test. **Results.** Of the 60 patients included in our analysis, 29 received best supportive care, 20 received azacitidine based therapy and 11 received intensive chemotherapy with curative intent. At a median follow up of 7.2 months (range: 0.5 to 26.4 months), estimated median OS for patients who received azacitidine based therapy was 10.9 months (range: 2.4 to 22.5 months), compared with 8.9 months (range: 0.9 to 26.4 months) for patients who received intensive chemotherapy (p = 0.89) and 2.4 months (range: 0.5 to 9.5 months) for patients who received best supportive care (p<0.005). Compared with azacitidine based therapy, intensive chemotherapy is associated with more inpatient days (median 99.5 (range: 27 to 150 days) vs 46 (range: 7 to 142 days) days; p=0.017) and higher number of episodes of febrile illness requiring inpatient stay or intravenous antibiotics (median 3 vs 2; p=0.019). Amongst patients who received azacitidine, those with bone marrow blast counts of less than 30% achieved a longer median survival compared with those with bone marrow blast counts of 30% or more (14.6 vs 9.8 months), however the difference was not statistically significant (p=0.31). Standard and favorable risk cytogenetics is associated with better survival compared with high risk cytogenetics in patients receiving azacitidine (14.6 vs 8months; p = 0.028). **Conclusions.** Compared with intensive chemotherapy in elderly patients with AML, azacitidine based therapy is associated with statistically similar median survival but lower number of hospitalization days and infective episodes, potentially maximizing time in the community while possibly maintaining a relatively good quality of life.

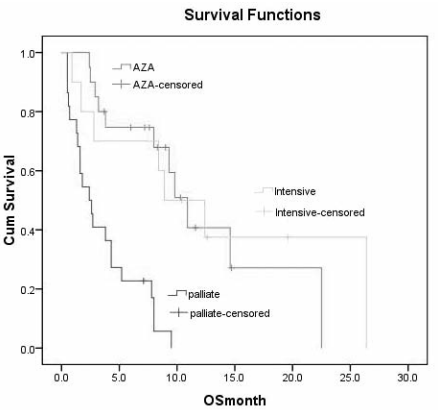

Figure 1. Kaplan Meier survival curve.

0658

PRE-CLINICAL CHARACTERIZATION OF ANTI-CXCR4 ANTIBODY, BMS-936564/MDX-1338, AND PHASE 1 CLINICAL EXPERIENCE IN PATIENTS WITH RELAPSED/REFRACTORY AML

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BMS-936564/MDX-1338 is an IgG4, fully human monoclonal antibody that specifically recognizes human CXCR4 and is currently in Phase 1 studies in patients with relapsed/refractory acute myeloid leukemia (AML) and multiple myeloma (MM). CXCR4 is a seven-transmembrane, G-protein-coupled receptor in the CXC chemokine receptor family. Expression of CXCR4 is elevated in a variety of cancers and the interaction of CXCR4 on tumor cells with CXCL12 in the bone marrow promotes tumor cell survival and growth. We have developed an antibody targeting CXCR4 which is predicted to be efficacious in a number of hematopoietic malignancies. Here we describe the pre-clinical *in vitro* and *in vivo* characterization of BMS-936564 and the preliminary clinical experience with BMS-936564 in a Phase 1 first-in human study in patients with relapsed or refractory AML.In vitro studies demonstrate that BMS-936564 binds to CXCR4 expressing cells with low nanomolar affinity. The antibody blocks CXCL12 binding to CXCR4 expressing cells and inhibits CXCL12 induced migration and calcium flux with low nanomolar EC50 values. In addition to these activities, BMS-936564 induces apoptosis on a large number of hematopoietic cell lines. Investigations into the pathway of apoptosis identified changes in mitochondrial membrane permeability and caspase 9 activation suggesting that the antibody is mediating apoptosis through the intrinsic pathway. Interestingly, the apoptotic activities associated with the CXCR4 antibody are CXCL12 independent. When given as monotherapy on established tumors, the antibody exhibits anti-tumor activity in multiple AML, NHL and MM xenograft models.In the Phase 1, first-in-human, multiple ascending dose clinical study in patients with relapsed or refractory AML, BMS-936564 is initially given as monotherapy during a lead-in period, followed by its administration with chemotherapy. The primary objectives are to assess safety and tolerability and to determine maximum tolerated dose. The data presented will include preliminary safety, pharmacokinetics, pharmacodynamics, and clinical efficacy. Informed consent has been obtained for all study subjects.

0659

ANALYSIS OF PROGNOSTIC FACTORS FOR SURVIVAL OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Acute myeloid leukaemia (AML) occurring in people aged 60 years and older is a distinct disease with an unfavourable prognosis owing to particular biological and clinical features. **Aims.** Through the application of different risk factor scores, we aimed to identify a subset of elderly patients who might benefit from intensive chemotherapy and for whom it could be possible to assess prognosis. We also wished to determine our own prognostic score system. **Methods.** This single-center study involved 102 patients aged ≥ 60 years with nonpromyelocytic AML and a follow-up period of 65 months. We classified the patients into risk groups for overall survival (OS) using the following prognostic models (named from the authors of the cited references): 1) Malfuson et al.; 2) Rollig et al.; and 3) Wheatley et al.. We compared the outcome of our patients after stratification into risk groups using the named prognostic models in order to determine which model is most applicable for assessment of our patients. The following parameters were evaluated as risk factors: age, leukocytosis (≥3010^9/L), lactate dehydrogenase (LDH) more than 1.5 x upper limit of normal, CD34 expression (≥10%), ECOG performance status (<1 vs ≥2), cytogenetic risk group according to the Europian LeukemiaNet (ELN) and comorbidity score (<3 vs ≥3) obtained from the hematopoetic cell transplantation-specific comorbidity index (HCT-CI). Kaplan-Meier curves and log rank test were used to estimate OS and to compare differences between survival curves. For multivariate analysis a Cox proportional hazards model was constructed for OS adjusting for six variables. A backwards elimination procedure was employed to exclude redundant or unnecessary variables. Integer weights for the risk score were derived from Cox proportional hazards modeling. The prognostic score was validated via 10-fold cross validation. **Results.** The mean age of the patients was 67 years (range 60-89). For our group of patients the most significant coincidence with outcome was with Wheatley's scoring system, which stratified patients into three risk groups: favorable, standard and poor. Our results showed that the most significant factors for OS in univariate analysis were: age ≥65 years (p = 0.016), ECOG PS ≥2 (p = 0.004), leukocytosis (p = 0.046), elevated LDH (p <0.001), adverse cytogenetic (p <0.001) and HCT-CI ≥3 (p <0.001). Our prognostic scoring system was developed after analysis of the prognostic risk factors: age <65 years, normal LDH and HCT-CI <3 = 0 points, where age ≥65 years = 1 point, and elevated LDH and HCT-CI ≥3 = 2 points. According to this prognostic model, patients were classified into three risk groups: favorable = 0-2 points, intermediate = 3-4 points, and poor = >4 points. The OS between these groups was highly significant (p <0.001). Moreover, in comparison with other prognostic models by Cox analysis, our prognostic model had the greatest statistical significance: p<0.001, HR 2.013 (95% CI 1.464 - 2.768). **Conclusions.** The prognostic model developed in this study can distinguish between elderly AML patients in different risk groups regarding prediction of OS and can indicate a subset of patients suitable for intensive chemotherapy.

0660

USE OF HIGH DOSE CYTARABINE INDUCTION IN ADULT *de novo* AML IS NOT ASSOCIATED WITH IMPROVED SURVIVAL

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Background. High dose cytarabine (HiDAC) in treatment of adult AML is associated with improved disease free survival (DFS). However, data on the relative benefits of incorporating HiDAC into induction *versus* consolidation chemotherapy cycles is limited. **Aims.** We aimed to determine the outcome of adult patients with *de novo* AML treated with standard dose cytarabine *versus* HIDAC-based induction strategies at our institutions. **Methods.** All patients with *de dovo* AML aged 15-59yrs treated with induction chemotherapy at our institutions between February 1999 and July 2011 were retrospectively identified from institutional data bases. Information on cytogenetic risk (using Intergroup criteria), chemotherapy protocols, response to therapy, OS, and DFS were then determined retrospectively by review of individual medical records. Survival analyses were calculated by the Kaplan-Meier method and compared using the log-rank test. **Results.** In total 211 patients with *de novo* AML had been treated, including 105 patients (50%) treated with "standard"cytarabine at 100mg/m2 days 1-7, with 103 patients receiving standard 7+3 with idarubicin 12mg/m2 days 1-3 and 2 patients receiving standard 7+3+7 with etoposide; and 106 patients (50%) treated with HiDAC-based induction, including 76 patients treated with "Big ICE"(Blood 2005; 105: 481), 21 treated with "HiDAC 7+3"(HiDAC 3gm/m2 BD days 1, 3, 5 + 7 plus idarubucin 12mg/m2 days 1-3), 4 treated with FLAG and 5 with other HiDAC-based regimens. Median age of the entire cohort was 45yrs (range 15-59yrs). Based on cytogenetic profile, 36 patients (17%) had good-risk AML, 142 (67%) intermediate-risk and 33 (16%) poor-risk disease. Overall CR rate was similar between the 2 induction strategies (93% *vs.* 92% for standard *vs*. HiDAC based induction groups respectively; p=0.8). A majority of patients receiving standard-dose cytarabine induction subsequently received HiDAC-based consolidation (67%); conversely the majority of patients induced with HiDAC received standard-dose cytarabine in consolidation (81%). A similar proportion of patients in each group underwent allogeneic transplantation in 1st remission (34% *vs.* 25% for standard *vs.* HiDAC based induction groups respectively; p=0.17). At a median FU of survivors of 47mths (range 1-152mths), no difference in either OS or DFS was apparent between the standard and HIDAC-based induction groups (median OS 81 *vs*. 78mths respectively; p=0.9; median DFS not reached *vs.* 59mths respectively; p=0.9). Even when stratified into specific cytogenetic risk groups, no improvement in either OS or DFS was demonstrable with HiDAC-based induction. **Conclusions.** HiDAC-based induction for *de novo* AML in adults <60yrs of age is not associated with improvement in either OS or DFS in comparison with use of standard-dose cytarabine induction +/-y HiDAC based consolidation.

Acute myeloid leukemia - Clinical 4

0661

LIPOSOMAL DAUNORUBICIN, FLUDARABINE AND CYTARABINE (FLAD), A WELL TOLERATED AND EFFECTIVE TREATMENT BRIDGING TO STEM CELL TRANSPLANT IN 66 PATIENTS WITH RELAPSED-REFRACTORY ACUTE LEUKEMIA

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Background and Aims. Patients with relapsed or refractory acute lymphoblastic leukaemia (ALL), lymphoid blast crisis of CML (CML-LBC) and acute myeloid leukaemia (AML) experience a very poor outcome and only a small proportion of them can be rescued by allogeneic stem cell transplant (BMT). In these patients the use of Daunorubicin is usually limited by drug-induced cardiotoxicity depending on the cumulative dose administered. Compared to the unencapsulated antracycline, liposomal daunorubicin (DNX) is characterized by a higher tumor cell delivery and a reduced toxicity profile. We previously showed that the combination of fludarabine plus Ara-C and DNX (FLAD) had clinical activity in patients with poor risk AML and relapsed ALL (Clavio et al 2004). Herein we present our experience with FLAD as salvage and bridging therapy to BMT. **Patients and Methods.** The regimen consisted of three-days treatment with a 30-minute infusion of Fludarabine 30 mg/sqm followed 4 hours later by a 4 hour infusion of Ara-C 2 g /sqm and a 60 minute infusion of DNX 100 mg/sqm. All patients achieveing CR received at least an additional course of FLAD and, when possible, were scheduled for BMT. We have treated until now 34 pts with ALL, refractory (n. 12) or in relapse (n. 22, 4 after allogeneic BMT), 3 pts with relapsed CML-LBC, and 29 patients with relapsed (n. 20, 4 after BMT) or refractory (n. 9) AML. Median age was 34 years (range 13-76) for ALL patients and 53 years (range 13-70) for AML patients. Patients had received a median of 3 prior regimens (range 1-7). Seventeen ALL/CML-LBC patients (45%) and 7 AML patients (24%) had unfavourable cytogenetic alterations. **Results.** In ALL/CML-LBC group 4 pts died of infection during therapy (10%) and 23 patients out of 37 (62%) obtained complete remission (60% of those treated for relapse and 67% of refractory patients). In the AML group one patient died of infection during therapy (3%) and 14 patients out 29 (48%) achieved CR (70% and 0% among relapsed and refractory patients, respectively). CR rate was significantly affected by disease status (relapsed vs refractory) and karyotype in AML and by age in ALL patients. Fourteen ALL/CML-LBC patients (38%) and 8 AML patients (27%) underwent BMT. Projected DFS and OS for ALL patients is 9,1% and 13% respectively, both at 36 and 60 months. For AML patients DFS and OS is 10,3% and 10,7%, respectively, at 36 months and 60 months. DFS and OS were affected by age only in ALL (p 0.019) and by disease status (p 0.0001) and karyotype (p 0.03) in AML group.FLAD was well tolerated. Few severe infectious and no cardiac complications were recorded. **Conclusions.** Our experience in a series of patients with very severe prognosis shows that FLAD is a feasible and effective salvage treatment for patients with relapsed and refractory ALL and CML-LBC and relapsed AML. Furthermore, the low toxicity of the regimen and the fast haematological recovery allowed a significant proportion of patients (33% overall) to benefit of BMT.

0662

ROLE OF PERIPHERAL BLAST CLEARANCE IN SURVIVAL OF PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Background. A combination of cytarabine and an anthracycline is the standard therapy for patients with AML and is associated with a complete remission (CR) rate of 60-70%. Early clearance of peripheral blood (PB) blasts has been established as an important prognostic factor in acute lymphoblastic leukemia. **Aims.** We evaluated the dynamics of PB blasts and white cell count (WBC) clearance in patients with AML undergoing induction chemotherapy with AI (ara-C 1.5g/m2x3d and idarubicin 12mg/m2x3d) at our institution and their impact on long-term outcomes.Patients & **Methods.** We reviewed the dynamics of PB blasts (PB blasts=0%) and WBC (WBC≤0.1x109/dL) clearance in patients with AML receiving AI (n=149). Patients with acute promyelocytic leukemia were excluded. Besides PB blasts and WBC, patient characteristics studied include age, hemoglobin, platelet count, bone marrow blasts and cytogenetics. **Results.** Of the 149 patients included in the study, 105 (70%) were <60 years of age, median age at treatment was 55 years (range, 19-73), median WBC 4.9 (range, 0.3-97.4), platelets 39 (range, 2-581), hemoglobin 8.4g/dL (range, 3.3-13.2), PB blasts 15% (range, 0-95), BM blasts 45% (range, 1-96), diploid cytogenetics (n=54, 36%) and poor cytogenetics (n=57, 38%). The overall response rate (ORR=CR+CRp) was 65% (58%+7%). Forty-four (30%) pts were resistant to induction therapy. To evaluate the dynamics of PB WBC and PB blast clearance, we divided all pts in 5 groups depending on the day they cleared their WBC or blasts from PB: group 1 (0-1 days), 2 (2-3 days), 3 (4-5 days), 4 (6-8 days), and 5 (beyond day 8). No differences in CR rates were observed across all 5 groups according to the time of clearance of PB WBC (p=0.65). Likewise, similar median overall survival (OS) rates were observed in all 5 groups regardless of the timing of PB WBC clearance (p=0.25). When groups 1, 2 and 3 were merged and compared to the merger of groups 4 and 5, the OS remained similar (p=0.30). However, the timing of PB blast clearance was associated with a distinct probability of achieving CR in all 5 groups (p=0.003). When groups 1, 2, and 3 were merged and compared with the merge of groups 4 and 5, the ORR for the resulting two new groups was 93% and 7% (p<0.001) and the corresponding median OS was 40 weeks and 75 weeks (p=0.038) suggesting that early PB clearance predicts long-term outcomes. In the Cox regression model,in addition to cytogenetics and platelet count, PB blasts clearance was also noted to be significantly associated with event free survival (p=0.03). However when controlling the association between PB blast clearance and response and PB blast clearance and survival, the association disappeared (p=0.19).Conclusion:We have shown in a large cohort of uniformly treated pts with AML that early clearance of PB blasts (but not WBC) is an important risk factor for achievement of CR and for OS. Clearance of PB blasts should be considered in prognostication schemas for pts with AML.

0663

CYCLIC AMP PROTECTS MYELOCYTIC LEUKEMIA BLASTS AND THE APL NB4 CELL LINE AGAINST ANTHRACYCLINE-INDUCED APOPTOSIS VIA ACTIVATION OF PKA TYPE I

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Background. Cyclic AMP is the second messenger for a number of extracellular signals, including endogenous and exogenous beta-adrenergic stimulators and members of the prostaglandin family. While cAMP protects mature neutrophils against death, its effects on hematopoetic cell growth and survival are pleiotropic (1). A striking effect of cAMP is to enhance the ATRA-induced maturation of acute promyelocytic leukemia (APL) cells, like the NB4 cell line (2). ATRA-induced maturation is a cornerstone in APL therapy, and its combination with cAMP signaling stimulators has been advocated to improve current APL therapy. **Aims.** We wanted to study how the cAMP level modulates the response to current therapy for AML and APL. **Methods.** Blasts from AML and APL patients and several cell lines (NB4, HL60, MOLM13) were tested for death induction by DNR in the absence and presence of cAMP elevating agents (PGE2 + PDE inhibitor or cAMP analogs). cAMP analog pairs specifically activating cAMP-dependent protein kinase I (PKA-I) or cAMP-dependent protein kinase I (PKA-II) were used to elucidate the major mediator of the protection. **Results.** We have recently reported that cAMP synergizes with the first line drug daunorubicin (DNR) to induce apoptosis in the AML cell line IPC-81 (3). In patient blasts, we found no instance of cAMP enhanced DNR-induced apoptosis. Rather, we noted that cAMP protected the majority of the patient blast isolates against DNR-induced death. While no such protection was noted in HL60 or MOLM13 cells, protection by cAMP elevation was pronounce also for the NB4 APL cells. We found that cAMP protected NB4 cells against DNR (and Idarubicin) both in the absence and presence of ATRA and that the uptake and nuclear accumulation of anthracycline was unaffected by cAMP. The major mediator of the protection was PKA-I: Protection was achieved by PKA-I activating, but not PKA-II directed cAMP analog pairs. It was nearly abolished by the PKA-I directed inhibitor Rp-8-Br-cAMPS, but not by stable RNAi-induced downregulation of PKA-RII. cAMP mediated protection was independent of p53 status and mTOR pathway activity. The protection was associated with inactivating phosphorylation of the pro-apoptotic protein Bad and activating phosphorylation of the AML proto-oncogene CREB, both on known PKA targeted residues. **Conclusions.** The antagonism between cAMP and anthracyclines represents a therapeutic dilemma. In current APL therapy an anthracycline is combined with the maturation-inducing agent ATRA. ATRA depends on cAMP to achieve optimal differentiation, and stimulation of cAMP signaling has been advocated to improve the ATRA effect in APL patients. The present findings suggest that cAMP stimulation could be counter-therapeutic together with anthracyclines, and that cAMP elevating drugs like phosphodi-

esterase inhibitors should be used with caution during anthracycline treatment.

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0664

HIGH RESPONSE RATE TO COMBINED THERAPY OF LOW DOSE GEMTUZUMAB OZOGAMICIN AND CYTARABINE IN ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS WITH FAVORABLE AND INTERMEDIATE I RISK CYTOGENETICS

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Introduction. The management of older patients with acute myeloid leukemia (AML), where the median age of presentation is approximately 70 years, remains a major challenge. The gemtuzumab ozogamicin (GO), a humanized anti-CD33 monoclonal antibody conjugated with an antitumor antibiotic, has been shown to be effective agent in the treatment of relapse AML in elderly. Two years ago, the FDA recommended the removal of GO from the US market due to product's safety issue and limited benefit in induction therapy of young (< 60 years) newly diagnosed AML patients. However, recently two prospective randomized trials showed a significant improvement in overall survival to the addition of GO to induction therapy in elderly patients with AML. The GO dose and the time of administration as well as the chemotherapy protocol were different. The aim of this study was to evaluate the safety and the efficacy of low dose GO with cytarabine in elderly patients with newly diagnosed AML. **Results.** Over the past five years, we have treated 16 elderly AML patients [median age 72 years (range 64-82)] with GO (3 mg/m^2) followed by continuous infusion of cytarabine (100 mg/m^2) for 7 days. Consolidation therapy was not predefined. The cytogenetic risk groups were defined using the new Genetic Risk Classification of the European LeukemiaNet Recommendations.Complete remission (CR) was achieved in 68.8% of patients however, only in patients with favorable or intermediate I cytogenetic risk groups. Of the 12 patients with favorable and intermediate I genetic group AML, 11 (91.7%) achieved CR. By comparison, of all 4 patients with intermediate II or adverse genetic groups, none of the patients achieved CR (p=0.003). The median disease-free survival and overall survival was 10.9 months and 18.8 months, respectively, for patients who achieved CR. The estimated median survival was 15 months in the favorable and intermediate I cytogenetic groups and only 4.4 months in the intermediate II and unfavorable risk groups (p=0.008). The toxicity profile was also manageable in AML patients mainly older than 70 years in a good performance status. The eight-week mortality was 6.25% (1/16), which is relatively low in this high risk group of patients. **Conclusions.** These data are in line with results from two randomized trials showing that the addition of low dose GO should be considered as first line induction therapy in selected elderly patients and raises the question regarding the optimal combined chemotherapy.

0665

CAN MDR1 GENE POLYMORPHISMS IMPROVE THE PROGNOSTIC IMPACT OF WHO ACUTE MYELOID LEUKEMIA CLASSIFICATION?

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Backgroud. Acute leukemias are clonal malignant disorders characterized by aberrant proliferation, differentiation and maturation of leukemic progenitors and precursor cells. Classification of these diseases, previously based only on morphological and cytochemistry findings (FAB classification), now also includes phenotypic aspects as well as cytogenetic and molecular characteristics, known to be valuable prognostic factors (WHO classification). Primary drug resistance in acute leukemia is probably the major cause of treatment failure and death. Many mechanisms have been thought to be related with this event, being the expression of P glycoprotein, the product of MDR1 gene, one of them. This protein functions as an efflux ATP-dependent pump, clearing drugs (such as antracyclines, vinca alkaloides, taxanes) out of cancer cells. **Aims.** to define the prevalence of MDR1 genes polymorphisms in Portuguese patients with acute myeloid leukemia (AML) and to evaluate the relevance of these polymorphisms in disease outcome (Overall Survival - OS). **Materials and Methods.** from 315 patients diagnosed and treated in our institution with AML we studied 25. DNA was extracted from peripheral blood samples. The polymorphism C3435T and C1236T was genotyped by Real Time PCR (RT-PCR) with Taqman®SNP Assay genotyping C___7586857_20 and C___7586662_10, respectively, from Applied Biosystems. Statistical analysis was performed with SPSS®Statistics v17. **Results.** Nineteen patients were males, median age 54 years old with good performance status (ECOG 0-1 in 84% patients). According to WHO classification 8 patients (32%) had AML with recurrent genetic abnormalities, 7 patients had AML with myelodysplasia-related changes and AML nos (28% each) and 12 % (n=3) had therapy related AML. Nine patients were treated according to SWOG9126, 8 patients according to "7+3"regimen, 4 patients were treated with mitoxantrone and arac and 2 patients were treated with acute promyelocytic protocols.In polymorphism C3435T the presence of C in both alleles was found in 9 cases (36%) and the presence of one allele T was observed in 16 cases (64%). In the other polymorphism (C1236T) 10 cases (52%) had two alleles C and 10 cases (40%) had one allele T. In none of the studied polymorphisms the presence of both alleles T was identified.The OS at 3 years for all patients was 40%. No significant difference was found in cytogenetic or age subgroup analyses of OS. The studied polymorphisms did not interfere in OS.A statistically significant difference (p=0,012) was found in the OS at 3 years, according to WHO subgroups in the presence of allele T cariers, which could not be reached when all patients were analyzed. Once again no significant difference was found when other subgroups analyses were performed (therapeutic, cytogenetic or age). **Discussion and Conclusions.** according to our results the WHO risk stratification is improved when adding to the subgroup analyses the evaluation of MDR1 polymorphisms. The relevance of WHO subgroups achieve more impact when allele T is present in C1236T. The treatment with SWOG 9126 regimen, which includes cyclosporine, did not improve outcome.Inclusion of more patients is a goal to reach in a near future aiming to ameliorate results.

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A SINGLE INSTITUTION EXPERIENCE OF INDUCTION WITH HIGH DOSE CYTARABINE AND MITOXANTRONE FOR HIGH RISK ELDERLY ACUTE MYELOID LEUKEMIA (AML) PATIENTS INCLUDING AGE > 70

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Background. Patients with high risk AML, defined as age>60 or presence of high risk cytogenetics carry a poor prognosis and inferior outcomes after standard 7+3 induction chemotherapy. Reported CR rates have been as low as 6% and induction death rates as high as 48%. **Aims.** We present here our experience on treating high risk AML patients with an induction regimen consisting of high dose mitoxantrone and cytarabine. **Methods.** We performed a retrospective analysis of all patients with AML who received this induction regimen from January 2009 to December 2011 at our institution. Patients treated with this regimen included patients who were age > 60 and age <60 if they had received prior treatment with daunorubicin, or treatment during daunorubicin shortage. Each patient received high dose cytarabine at 3gm/m2 over three hours on days 1 to 5 and mitoxantrone 60-80mg/m2 once on day 2. The primary endpoints of the study were response (CR + CRp) at day 30, treatment related mortality (TRM) within 30 days of initiation of treatment, overall and progression free survival (OS & PFS), ability to proceed to transplant and outcomes after transplant. Impact of high risk cytogenetics and non-denovo AML on the outcomes was analyzed and a subset analysis of patients age >70 was performed. **Results.** 50 AML patients received this induction regimen. The median age was 66.5 years (28 - 83), 20 patients (40%) were age ≥ 70. Median age adjusted Charlson comorbidity index (CCI) was 6 (2-12). Other high risk features included high risk cytogenetics in 27 (54%) and non-denovo AML in 24 (48%). 70+ subset were less likely to be denovo (p=0.0481). Response (CR+CRp) rate was 88% (44 of 50) including 11 who had CR with incomplete platelet recovery (CRp). Six (12%) patients were refractory to induction. There was no treatment related mortality. The median OS was 31.9 months (7.4 -*) Median PFS was *not reached (9.4 -*). Twenty five (50%) patients were able to proceed to autologous (6) or allogeneic (17) or autologous followed by allogeneic (2) stem cell transplantation. The median time to transplant was 105 days (32 to 195). Median OS and PFS of the patients who underwent SCT was *not reached (Figure 1). Median time to neutrophil and platelet recovery was 26 days (19-43) and 27 days (9-44) respectively. Echocardiography was performed either for cardiac symptoms or as pre transplant work up. Cardiac toxicity was noted in 14 (28%) patients, at a median of 63 days (22 -139) after induction chemotherapy. Anthracycline induced cardio toxicity was not reported to be the cause of death in any of these 14 patients. **Conclusions.** In this high risk AML population, high dose mitoxantrone and cytarabine induction was well tolerated and demonstrated a response (CR+CRp) rate of 88% and no induction deaths. Half (50%) were able to proceed to SCT. Advanced age

or high risk cytogenetics or non denovo AML did not adversely affect response rate or PFS, thus highlighting the utility of this regimen in high risk newly diagnosed elderly patients with AML.

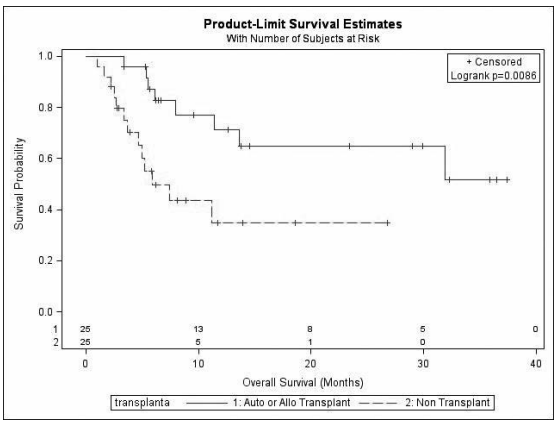

Figure 1 Overall Survival of Transplant versus Non transplant patients.

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COMPASSIONATE USE OF CLOFARABINE IN ACUTE MYELOID LEUKEMIA IN SPAIN: A RETROSPECTIVE STUDY

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Introduction. Clofarabine is a second generation purine nucleoside analog approved by FDA and EMA in refractory paediatric ALL patients. Phase I and II studies have reported Clofarabine activity in AML and has emerged as a potential alternative for relapsed and refractory patients with AML as well as unfit patients. **Aims.** The aim of this study is to evaluate the experience in Spain with Clofarabine in the treatment of adult AML patients, analyzing effectiveness and toxicity profile. **Methods.** Twenty Spanish sites participated in this retrospective study of adult patients with AML treated with Clofarabine by compassionate use. Clinical records were reviewed collecting demographic date and the use of Clofarabine. Main points were complete remission as IWRv2003 criteria and toxicity as CTCAE v3.0 of NCI scale. **Results.** Between July 2007 and August 2011 a total of 78 AML adult patients were treated with Clofarabine based chemotherapy in Spain. We obtained clinical data from 73 cases. Median age at Clofarabine treatment 52.3 years (18-77). Male/Female: 36/37. Previous Myelodisplastic syndrome 13 (17.8%). Cytogenetic data was available in 67 patients (91.7%), and 31 of them had high-risk cytogenetic. Twelve of these patients had had a previous ALO transplant.Clofarabine treatment: 65 patients received CLF as salvage therapy (untreated AML relapsed in 27 cases and refractory disease in the rest 38), median previous lines 3 (1-7). The remaining 8 patients received Clofarabine as front-line treatment. Clofarabine was administered in combination with AraC in 95% patients and 93% received a five days schedule. The most frequent Clofarabine dose/day were: 40 mg/m^2/day (47 patients), 30 mg/m^2/day (11 patients), and 20 mg/m^2/day (10 patients).Response and outcome: Twenty three (31.5%) patients achieved complete remission and 38 (52%) had resistant disease.The statistical analysis shows a significant difference in the CR rate between first line therapy (CR 62.5%) and savage therapy in relapsed (CR 44%) and salvage therapy in refractory (CR 18%), (p0.012). Neither adverse cytogenetics nor previous MDS and previous Alo transplant did influence CR rate (p0.56, p0.54 and p0.53 respectively). Toxicity: All patients presented grade IV hematological toxicity with grade IV neuthopenia, grade IV trombopenia and transfusion depend anemia. The incidence of extra hematological toxic effects were low, creatinine >3 mg/dL: 5 cases (6.5%); bilirrubine > 3 mg/dL: 11 cases (14%). Twelve (16.4%) patients died during induction. **Conclusions.** In this study Clofarabine showed clinical activity in adult AML patients and confirms the effectiveness of Clofarabine as a salvage regimen. Of note was that Clofarabine could potentially overcome some adverse known factors as cytogenetics.

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THE CLINICAL SIGNIFICANCE OF THE EXPRESSION OF MDR-1, MRP-1. BCRP AND LRP MRNA IN ADULT ACUTE MYELOID LEUKEMIA

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Background. Multidrug resistance (MDR) is a clinically relevant problemin the treatment of acute myeloid leukemia (AML). Whilesome mechanisms of drug resistance have been well characterized,this phenomenon still remains incompletely understood. One mechanism behind drug resistance is altered drug transport, which could be due to over-expression of the transport proteins that function as a membrane pump responsible for the efflux of a range of chemotherapeutic drugs: p-glycoprotein (Pgp)-product of *MDR1* gene and multidrug resistant protein-1 (MRP-1), breastcancer resistance protein (BCRP), lung resistance-related protein (LRP) encoded by the relevant genes. **Aims.** The aim of this study was to analyze the expression of the *MDR-1, MRP-1, BCRP, LRP* messenger RNA (mRNA) in relation to the response to induction chemotherapy and relapse and with pretreatment laboratory and clinical characteristics and prognostic factors. Moreover, the influence of MDR genes on disease free survival (DFS) and overall survival (OS) were estimated in presented patients. **Methods** A total of 185 adult consequent patients (88 females and 97 males) at a median age of 53,1 years (range18,5-86,6 years) with newly diagnosed, previously untreated AML, as well as 40 healthy donors were included in this study. DNA and RNA were extracted from mononuclear cells of bone marrow (147 patients) or peripheral blood (38 patients) samples collected after approval from the ethics committee and informed consent from the patients. *FLT3*-ITD was detected with PCR method in 53 patients (29%). The RQ-PCR method was performed for the assessment of expression ofthe *MDR-1, MRP-1, BCRP* and *LRP* mRNA and the results were presentedas coefficients calculated with an intermediate method accordingto Pfaffl's rule.The relative expression ratio of a target gene was calculated based on efficiency and the Ct deviation of the healthy donor´s sample versus the patient´s sample, and expressed in comparison to a reference gene: **Results.** The high expression of MDR genes correlates with worse treatment outcome of patients with AML. In univariate analysis, the high expression of *MDR-1* mRNA (>0,131) was associated with outcome of induction therapy (p=0,06) and of *BCRP* mRNA (> 1,148) with high relapse rate (RR) (p=0,013). We found that high expression of *MDR-1* (>0,131), *MRP-1* mRNA (> 0,84) and *BCRP* mRNA (> 1,148) significantly influence DFS (p=0,059, 0,032 and 0,009, respectively) and OS (0,048, 0,014 and 0,059, respectively). The *LRP* mRNA expression had no impact on treatment outcome in AML. Interestingly, high *BCRP* mRNA expression (>1,148) proved to be an independent prognostic factor for RR (p=0,011) and DFS (p=0,002) in the multivariate analysis.**Conclusions** The combined analysis of MDR genes allowed different classes of AML to be identified, with distinct clinico-biological features. Our study shows that MDR genes expression should also be considered for risk-adapted treatment strategies in AML in the future. It seems that especially BCRP is associated with clinical resistant disease in AML and influence the RR and DFS.This project was sponsored by grant of The Ministry of Science (NN402208935).

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THE DIFFERENCES IN PROGNOSTIC OUTCOME BETWEEN AML1/ETO AND CBFβ/MYH11 POSITIVE AML PATIENTS

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Background. CBF-AML is considered to be a good-risk subtype of AML. However, there might be differences within this subgroup according to the respective fusion gene as well as due to various secondary aberrations. The results published so far are quite inconsistent. **Aims.** To search for possible differences in clinical outcome between patients with *AML1/ETO* and *CBFβ/MYH11* fusion genes. **Patients and Methods.** CBF-AML was diagnosed in 49 patients, their median age being 38.7 (18.5-70.6) years. The male/female ratio was 34/15, median follow-up was 38 months. RNA from 46 of them was available for searching of secondary aberrations. Minimal residual disease (MRD) was prospectively followed up in 39 patients.Presence of *AML1/ETO* and *CBFβ/MYH11* was detected by RT-PCR; MRD was monitored using real time RT-PCR. Direct sequencing was used to identify patients carrying secondary alterations such as *FLT3*/ITD, *FLT3* D835, *C-KIT* or *K-RAS* mutations. **Results.** 27 patients had *AML1/ETO,* 9/26 (34.6%) harboured secondary aberration (2 had *FLT3*/ITD, 2 *FLT3* D835, 4 carried *C-KIT* and 1 *K-RAS* mutations). 22

patients were *CBFβ/MYH11* positive, 13/20 (65.0%) had another molecular lesion (2 had *FLT3*/ITD, 5 *FLT3*/TKD, 7 *C-KIT* and 2 *K-RAS* mutations), 2 patients carried more than one of these changes. Secondary aberrations were more frequent among *CBFβ/MYH11* patients (P=0.020).The initial median WBC count was 15.8 x 10^9/L (1.3-256.1 x 10^9/L) and it differed between the *AML1/ETO* and *CBFβ/MYH11* cases (12.8 vs. 52.5 x 10^9/L; P=0.004). *CBFβ/MYH11*-positive patients harbouring any of secondary molecular aberrations had higher WBC (105.3 vs. 30.5 x 10^9/L; P=0.081).All but 3 patients (all of them having *AML1/ETO*) reached complete remission (CR). 12 of 20 (60.0%) patients carrying *AML1/ETO* achieved molecular remission (MR; defined as MRD negativity in at least two following bone marrow samples, with a minimal interval of 2 months), while only 7/16 (43.8%; P=0.166) *CBFβ/MYH11*-positive patients reached MR. Reaching MR was unfavourably influenced by the presence of any of the secondary aberrations monitored (P=0.023). Relapse free survival (RFS) was longer in cases reaching MR (P<0.0001) and there was no difference between *AML1/ETO* and *CBFβ/MYH11* cases. Only 1/19 (5.3%) patients reaching MR relapsed, while 9 relapses among 15 patients (60.0%) without MR were observed (P<0.0001). Patients achieving MR had also significantly longer overall survival (OS; P=0.025).*CBFβ/MYH11*-positive patients more often relapsed than those with *AML1/ETO* fusion (63.6% vs. 20.8%; P=0.002) and their RFS was shorter (P=0.014). The incidence of relapse as well as RFS was influenced by the presence of the secondary aberration only within the *CBFβ/MYH11*-positive subgroup (P=0.055 and P=0.033, respectively), not in the *AML1/ETO* patients (P=0.300 for relapse rate and P=0.603 for RFS). Although OS was not affected by the particular fusion gene within the whole group (P=0.878), when patients not reaching CR were excluded, there was a trend to shorter post-remission survival in *CBFβ/MYH11* cases (P=0.129). **Conclusions.** In our CBF-AML patient cohort, *CBFβ/MYH11*-positive cases had shorter RFS and higher incidence of relapse than those carrying *AML1/ETO* fusion. This might result from lower MR rate caused probably by higher incidence of the secondary molecular aberrations.

0670

PROGNOSTIC FACTORS OF ATRA RELATED COMPLICATIONS DURING INDUCTION TREATMENT OF APL

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Background. The combination of all-trans-retinoic acid (ATRA) and chemotherapy has made acute promyelocytic leukemia (APL) a highly curable leukemia. However, several complications are reported with this treatment the most serious and life threatening being the differentiation syndrome (DS). We aimed at identifying factors that could predict complications caused by ATRA during induction treatment of APL. **Results.** Fifty one patients with confirmed APL (by t (15,17) and/or PML/RARA) treated in our institution (University hospital of Tunis) between 2004 and 2010 using the Spanish PETHEMA LPA99 trial. Induction regimen consisted of ATRA 45 mg/m^2/d until CR combined to Idarubicin 12 mg/m^2 d2, 4, 6, 8 IV. Prednisone (0.5 mg/kg d1-d15) was added if WBC >10x 10^9/L to prevent DS. Median age was 30 yr (range 4-71), M/F was 0.64 , median WBC was 4.4x10^9/L(range 0.6 - 123). Additional cytogenetic abnormalities were seen in 17 patients (39.5%). Median body mass index (BMI) was 23.5 kg/m^2 (range 13- 40), BMI >35 was noted in 5 patients (3 F and 2 M). 43 patients achieved CR (86%). Seven patients (14%) had early death: 3 from CNS bleeding and 4 from DS.Complications due to ATRA were: DS (16 patients), scrotal ulcerations (4 patients), Sweet syndrome (1 patient), headache (12 patients), skin dryness (22 patients) and arthralgia (8 patients). Prognostic factors for complications of ATRA excluding DS were: BMI >35 (P=0.045), Platelet count > 40x10^9/l (P=0.045) and age > 20 y (P<0.001), whereas sex, WBC and morphology were not predictive. For DS, BMI >30 (P=0.029), renal failure (P=0.021) and WBC > 10x10^9/l (P=0.047) were independent predictor factors. With median follow up of 50 months EFS and OS were 74% and 78%, respectively. ATRA related complications had no significant impact on CR, EFS and OS. **Conclusions.** We found very high BMI (>35) and age over 20 years to be significantly associated with ATRA related complications (DS excluded) during induction treatment for APL.

0671

CD56 ANTIGEN EXPRESSION IN ACUTE MYELOID LEUKEMIA

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Background. The expression of CD56 antigen has been reported in several hematologic malignancies, including acute myeloid leukemia (AML). Evidence suggested that CD56 positivity could be associated with extramedullary leukemic infiltration and an unfavorable prognosis in AML patients, in particular in AML with t(8;21) and acute promyelocytic leukemia (APL). In other AML subtypes its role is less clear. **Aims.** To investigate the frequency, clinical and biologic characteristics and prognostic impact of CD56 expression in AML patients. **Methods.** Two hundred and five patients with AML (53 with APL) received intensive chemotherapy in our center between January 2005 and March 2011. Immunophenotypic analysis was available for all patients and CD56 positivity was considered when at least 20% of blasts expressed this antigen. Patients were treated with EORTC Protocols AML12/AML17, ATRA-IDA or AIDA GIMEMA protocol for APL and 2 patients with ELAM-02 pediatric protocol, followed by consolidation chemotherapy and allogeneic or autologous transplantation. **Results.** Median age was 54 years (range, 16-78) and 56.1% (n=115) of patients were females. Thirty eight (18.5%) patients were CD56 positive. Its frequency was significantly higher in FAB subtype M5 (p=0.05) and in patients with t(8;21) (p=0.000). CD56+ patients had a significantly higher median leukocyte count (17.5x10^9/L vs. 5.6x10^9/L, p=0.03), higher median *lactate dehydrogenase* (LDH) (p=0.05) and extramedullary infiltration (50% vs. 28.7%, p=0.01). CD56 positivity did not correlate with age, sex and blast count. To assess the prognostic significance of CD56 positivity, we excluded patients with more than 60 years (n=55) and patients with favorable cytogenetic - t(8;21) (n=4), inv(16)/t(16;16) (n=5) and t(15,17) or PML-RARA+ (n=53). We analyzed 88 patients (54.5% female), with a median age of 46 years (range, 16-60 years). Seventeen patients were CD56 positive (19.3%). The median follow-up after beginning of treatment was 16 months (range, 0.36-72 months). CD56 positivity did not influence complete remission (CR) rate. The 5-year Overall Survival (OS) was 27% (95% CI, 25.72-28.28) for CD56+ patients and 42.9% (95% CI, 42.15-43.65) for CD56- patients (p=0.19). The 5-year Disease Free Survival (DFS) was 21% (95% CI, 19.73-22.27) for CD56+ patients and 41.2% (95% CI, 40.47-41.93) for CD56- patients (p=0.34). In multivariate analysis, only older age and chemotherapy resistance were independent prognostic adverse factors in OS. **Summary and Conclusions.** Our data confirm that CD56 positivity is associated with FAB subtype M5, AML with t(8;21), higher median leukocyte count and LDH and extramedullary disease. However, no statistically significant results were found in CR, OS and DFS rates, despite our CD56+ patients showed a trend to have lower DFS and OS. These results may allow to hypothesize that stronger prognostic factors abolish the prognostic value of CD56.

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PROGNOSTIC FACTORS AND OUTCOME OF CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Translocation t(8;21) and inversion (inv)(16)/t(16;16) disrupt core binding factor (CBF) in acute myeloid leukemia (AML) and occur in approximately 7% and 8% of adults with *de novo* AML, respectively. CBF-AML has been associated with a favorable prognosis, but it seems that these two cytogenetic subgroups have different biological and clinical standpoints. **Aims.** The aim of this study was to assess prognostic risk factors for rate of complete remission (CR), overall survival (OS) and disease-free survival (DFS) in patients with CBF-AML. **Methods.** Fifty two patients with CBF-AML, 28 with t(8;21) and 24 with inv(16), diagnosed and treated during a 10-year follow-up period (2001-2011), were retrospectivelly assessed. The following parameters were estimated as risk factors for CR, OS and DFS: age, leukocytosis ≥50x10^9/L, CD117 expression ≥20% on leukemic cells assessed by flowcytometry, dysplastic features in bone marrow cells, hepatomegaly (>14 cm) and splenomegaly (>12cm). Patients were treated with the Medical Research Council (MRC) 10 regimen. C-kit assessment by PCR and treatment with MUD alogenous transplantation were not available. Risk factors were identified using univariate and multivariate analysis. **Results.** The mean age of the patients was 46 years (range 18-73). Median OS was 8 months, CR rate was 69.6%, relapse rate was 32,2% while DFS was 18 months. Significant risk factors for a lower CR rate in univariate analysis were: hepatomegaly (p=0.034), splenomegaly (p=0.015) and leukocytosis (p=0.001), while multivariate analysis indicated leukocytosis as the most significant risk factor (p=0.035). Significant risk factors for poor OS by univariate analysis were: age >50 years (p=0.012), leukocytosis (p=0.035) and CD117 antigen expression (p=0.022). Multivariate analysis identified leukocytosis as the most important risk factor for OS (p=0.006). Univariate analysis showed that significant risk factors for shorter DFS were: age >50 years (p=0.026), splenomegaly (p=0.011) and bone marrow dysplastic features(p=0.015). Moreover, the most important risk factor for DFS in multi-

variate analysis was bone marrow dysplasia (p=0.034). In the subgroup of patients with t(8;21), the overall CR rate was 80.8%, while in the subgroup with inv(16), it was 55%. In patients with t(8;21), the most important risk factor for CR rate (p=0.050) and OS (p=0.042) was age >50 years, while splenomegaly was the most important risk factor for DFS (p=0.050). In patients with inv(16), the most important risk factor for CR rate was leukocytosis (p=0.045), for OS it was age >50 years (p=0.040), and for DFS bone marrow dysplastic features (p=0.040). **Conclusions.** This study identified leukocytosis, age and bone marrow dysplastic features as the most important factors affecting CR rate, OS and DFS in patients with CBF-AML. These factors differ in importance between the two cytogenetic subgroups of CBF-AML, which indicates that patients with t(8;21) and inv(16) constitute two separate clinical entities.

0673

CLOFARABINE AS A SALVAGE THERAPY AND A BRIDGE TO TRANSPLANTATION IN PATIENTS WITH HIGH RISK ACUTE LEUKEMIA AND RELEVANT COMORBIDITY. A SINGLE CENTRE EXPERICENCE

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Backgroud. Acute myeloid leukemia (AML) is an aggressive hematological malignancy and the outcome of patients with relapsed/refractory disease still remains unsatisfactory. Clofarabine is a second generation nucleoside analog with efficacy in acute leukemia. Combination therapies of Clofarabine either with cytarabine arabinoside (ARA-C) in AML or with cyclophosfamide in acute lymphoblastic leukemia (ALL) are feasible and effective as induction therapy or salvage treatment. **Aims.** To determine the efficacy and safety of clofarabine as a salvage therapy and as a bridge to transplantation in frail patients with unfavourable cytogenetics relapsed/refractory acute leukemia. **Methods.** A retrospective analysis was conducted on 21 patients with acute leukemias treated in our Department. The diagnosis was made according to 2008 WHO criteria. Median age at diagnosis was 52 years (range 19-76); 12 (57%) patients with relapsed and 9 (43%) with refractory acute leukemias; AML (n: 19)/ALL (n: 2); patients were treated with a median of 2 prior regimens (range 1-5). Fifteen patients (71.4%) were considered at high, one intermediate and five (23.8%) low cytogenetic risk. Eighteen patients (86%) had significant comorbidity (48% had high risk Charlson score index). AML patients received salvage therapy with ARA-C 1 g/m^2 for 5 day and Clofarabine, 40 mg/m^2 for 5 days, from day 2 to day 6, whereas in ALL patients Clofarabine was combined with Cyclophosfamide 400 mg/m^2, given for 5 days. **Results.** Seven patients (33%) achieved complete remission (CR); among these, 3 (14%) were classified as high risk cytogenetic at the start of therapy. The median DFS was 110.5±179 days (median: 30; range: 3 - 740), while the EFS was 36.8±39.8 (median: 21; range: 3-150). Neither the DFS nor EFS were significantly associated to cytogenetic risk profile (p=0.96 e p=0.33, respectively). The DFS was correlated with EFS (Rho=0.61, p=0.003). Eight patients (38%), three in complete remission and three with persistence of disease, underwent transplantation. Two cases relapsed after allogeneic bone marrow transplantation (allo-BMT) and were treated with clofarabine as salvage therapy; the median survival from the beginning of therapy was 100 days (95% CI: 35-165). The median survival was significantly correlated with transplantation (log rank, p=0.03), resulting 157 days (95% CI: 86-228) in transplanted patients versus 58 days (95% CI: 0-134) in non transplanted patients. Side effects with clofarabine included nausea (49%), transient liver dysfunction (71%), skin rashes (43%), mucositis (31%). Patients were treated with antibiotic prophylaxis with fluoroquinolones. We documented 13 (61%) febrile neutropenia, 8 (38%) septic shock, 5 (23%) cardiac abnormalities (3 atrial fibrillation and 2 heart failure). **Conclusion**. Clofarabine in combination with ARA-C or Cyclophosphamide represents a promising combination in the treatment of acute leukemias both as a salvage therapy to induce remission and as a bridge to transplant.

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NK CELL AS THE THIRD LINEAGE OF MIXED PHENOTYPIC ACUTE LEUKEMIA: BOTH CD56 AND CD16 EXPRESSION IN ACUTE MYELOID LEUKEMIA IS SIGNIFICANTLY ASSOCIATED WITH THE HIGH NUMBER OF PLATELET

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Background. There is no consideration of natural killer (NK) cell lineage in defining of mixed phenotype acute leukemia (MPAL) according to the WHO 2008 classification as well as bilineal acute leukemia or biphenotypic acute leukemia (BAL) according to the European Group for the Immunologic Classification of Leukemia (EGIL) scoring system of previous WHO 2001 classification. CD56, one of the antigens expressed on NK cells, is commonly used for immunophenotyping of acute leukemia. CD56 expression in acute myeloid leukemia (AML) has been reported to be associated with poor prognosis, comparing of AML without CD56 expression. **Aims.** We hypothesized that CD56 expression of AML is not aberrant expression of AML but NK lineage expression as the 3rd lineage of MPAL. Immunophenotype analysis for CD16, granzyme B, and perforin together with CD56 as NK lineage markers were performed in AML, and the hematologic, immunophenotypic, and cytogenetic features were investigated according to the expression of NK lineage markers. **Methods.** Patients, who were diagnosed with AML and BAL according to EGIL scoring system from September 2007 through July 2009 and whose BM cells were available for immunophenotyping by flow cytometry, were reclassified according to the WHO 2008 classification. In addition of immunophenotyping analysis, we analyzed several clinical and biological characteristics: age, sex, complete blood cell count and blast count in peripheral blood, cytogenetic features, and AML risk status based on cytogenetics of National Comprehensive Cancer network (NCCN) guidelines. **Results.** A total of 117 patients diagnosed with AML and MPAL, were enrolled according to the WHO classification of 2008. One hundred three out of 117 patients were classified as AML, 14 patients were classified as MPAL, 11 as MPAL, B/myeloid and three as MPAL, T/myeloid. To compare the hematologic, immunophenotypic, and cytogenetic features according to the presence of the NK markers in AML, we categorized the 103 patients (91 AML and 12 MPAL, NK/myeloid) into three groups according to the expression of CD56 and CD16: patients with the expression of both CD56 and CD16 (12 patients, 11.6%), patients with the expression of CD56 or CD16 (42 patients, 40.8%), and patients without the expression of CD56 and CD16 (49 patients, 47.6%). The number of platelet was significant higher in the patients with the expression of both CD56 and CD16 than in the patients with the expression of CD56 or CD16 and the patients without the expression of CD56 and CD16 (P=0.011). The immunophenotypic features were similar, except for the expression of CD5 and CD117 (P=0.005 and P=0.034, respectively). No significant differences in other characteristics were observed between three groups. **Conclusions.** We showed the unique hematologic feature of AML with expression of NK markers, CD56 and CD16. Therefore, we suggest NK cell lineage as the 3rd lineage of MPAL and propose the additional MPAL subtype, namely, MPAL, NK/myeloid. Further studies will be required to ascertain the prognosis of MPAL, NK/myeloid and NK marker expression in AML should be considered in the treatment strategy.

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AZACITIDINE FOR TREATMENT OF MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIAS: A SINGLE CENTER EXPERIENCE

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Introduction. The efficacy of azacitidine in the treatment of high risk myelodysplastic syndromes (MDS), chronic myelomonocytic leukemias (CMML) and acute myeloid leukemias (AML) has been demonstrated. **Aims.** To investigate the outcome of patients receiving azacitidine in a daily clinical practice at our institution and its impact on disease response, overall survival (OS) and transfusions. **Materials and Methods.** We conducted this retrospective analysis on patients who received azacitidine between August 2005 and November 2011 at our institution for MDS or AML. The total number of treatment cycles, response rate, number of transfusions and overall survival were evaluated. **Results.** There were 96 patients, 59 (61%) males and 36 (39%) females with a median age of 70 years (25-91). The indication of azacitidine was the first line

treatment for MDS, mainly refractory anemias with excess of blasts,(n=68 (71%) patients: group1) and treatment following chemotherapy for AML patients (27 (29%) patients: group2). Prognostic factors according to FAB classification, ISPP risk and cytogenetics were assessed and will be further communicated. After a median follow-up of 9 months (1-75), the median number of azacitidine cycles in groups 1 and 2 was 8 (1-30) and 4 (1-16) respectively. There were 38 (40%) of responders (30 ingroup 1 and8 ingroup 2) while the rest of patients have progressed. There was a significant correlation between the number of treatment cycles and response rate; patients in response were statistically correlated with a number of cycles ≥ 6 (32 patients received ≥ 6 cycles:24 ingroup1 and8 ingroup 2). Accordingly, patients receiving ≥ 6 cycles of azacitidine had a significantly better survival comparing to those receiving less than 6 cycles. Beyond the number of 6 cycles, a higher treatment cycles did not show a significant difference in terms of OS. The median OS for the group 1 and 2 was 19.3 months (16-39) and 5.9 months (4.9-11.9) respectively, p<0.001. The median OS of responding patients was 24.5 months (24.5-not reached) while in the non-responders, it was 11.5 months (7-18.4). In terms of transfusion need, there was a significant decrease of red blood cells and platelets transfusions only in group 2, p=0.008. **Conclusions.** We showed a good response rate after azacitidine use especially when used for more than 6 cycles; its use in first line has significantly a better outcome on overall survival, while a transfusion benefit was demonstrated only in AML patients.

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PROGNOSTIC FACTORS IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background. Acute Promyelocytic Leukemia (APL) is nowadays considered the most frequently curable acute leukemia of adults. It is known that some prognostic factors can affect the outcome of APL patients. Recently, an increased body mass index (BMI) was associated with a higher risk of developing differentiation syndrome and disease relapse (Breccia et al, *Blood*. 2012) **Aims.** To investigate the clinical and biologic characteristics and prognostic factors in APL. **Methods.** Fifty-three patients with APL received intensive chemotherapy in our center between January 2005 and June 2011. Patients were treated with regimens based in All-*trans* retinoic acid and anthracyclins. The following prognostic factors were evaluated in the overall survival (OS) and disease free survival (DFS) analysis: sex, age, French-American-British (FAB) subtype, breakpoint PML, CD34 antigen expression, BMI, hemoglobin, leukocyte and platelet count at diagnosis. **Results.** Median age was 44 years (range, 18-78) and 62.3% (n=33) of patients were females. The breakpoint PML analysis results were known in 49 patients: 31 (63.3%) with BCR1-2 and 18 (36.7%) with BCR3. Ten patients (18.9%) were classified as microgranular variant of M3. These patients showed a significant association with BCR3 breakpoint mutation (p=0.05) and higher median D-Dimers (54.83 vs. 23.34, p= 0.003). Twenty-six patients (49.1%) were under/normal weight (BMI < 25 Kg/m^2) and 27 (50.9%) were overweight/obese (BMI ≥ 25 Kg/m^2). An increased BMI was associated with older age (p=0.000), but not with sex, FAB subtype, leukocyte count or breakpoint PML. With a median follow-up after beginning of treatment of 36 months (range, 0.07 - 78.9 months), the 5-year DFS was 54.4% (95% CI, 53.03-55.77) in overweight/obese and 80.3% (95% CI, 80.21-80.38) in underweight/normal weight patients (p=0.04). The 5-year DFS was significantly lower in patients with older age (44.2% for ≥44 years vs. 85.4% for <44 years, p=0.002), isoform BCR3 (43.8% for BCR3 vs. 87.6% for BCR1-2, p=0.01) and CD34 antigen expression (50% for CD34+ vs. 71% for CD34- patients, p=0.02). In OS analysis, only older age showed to significantly affect the outcome (p=0.001). In multivariate analysis, the independent prognostic factors with negative impact on DFS were: BMI ≥ 25 Kg/m^2 (hazard ratio = 8.18, p= 0.04) and isoform BCR3 (hazard ratio = 9.06, p=0.04). **Summary and Conclusions.** Despite the high curable rates in APL, some factors can adversely influence the outcome of these patients. In univariate analysis, increased BMI, BCR3, older age and CD34 antigen expression had a significant negative impact on DFS, but only BCR3 and increased BMI showed an independent prognostic impact in multivariate analysis. Our data support the recent interest of increased BMI as a strong outcome predictor in APL.

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VALIDATION OF THE EUROPEAN PROGNOSTIC INDEX FOR ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA IN THE FIRST RELAPSE

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Background. The outcome of patients with acute myeloid leukemia (AML) in the first relapse is poor. Predictive score for these patients could be generally applicable if they would furnish a simple and statistically valid prognostic score. **Aims.** The aim of this study was to assess the reproducibility of the European Prognostic Index (EPI) for stratification of patients with AML in first relapse into prognostic risk subgroups, as well as to evaluate various therapeutic options for treatment of these individuals. **Methods.** The single-center study involved 54 patients with nonpromyelocytic AML in the first relapse, with a 5-year follow up period. The patients were stratified into three prognostic groups: favorable, intermediate and poor, according the EPI. Four parameters were included in this index: length of relapse-free interval after first complete remission (CR), cytogenetics at diagnosis, age at relapse and whether prior stem cell transplantation had been performed. Patients were treated with three different therapeutic regimens: 1) Flagida - 45% of cases (Fludarabin 30mg/m^2 days 1-5; Cytarabin 2000 mg/m^2 days 1-5; Idarubicin 8 mg/m^2 days 1-3); 2) HiDAC - 35% of cases (Ara-C 3 gr/m^2 days 1,3,5) and 3) Mitoxantrone + Etoposide - 20% of cases (Mitoxantrone 10 mg/m^2 days 1-5; Etoposide 100 mg/m^2 days 1-5). Patients aged over 65 years were given palliative therapy (Hydroxyurea). The rate of second CR and OS were determined. **Results.** The mean age of the patients was 47 years (range 26-78). The overall rate of second CR was 40.9%. When patients were stratified into prognostic risk groups according to the EPI, the rates were as follows: favorable group - 100%, intermediate group - 41.7%, and poor group - 34.5%, which were significantly different (p = 0.05). Also, according the EPI stratification, OS (in months) was as follows: favorable group - 46, intermediate group - 17, and poor group - 13. Again, the differences were significant (p = 0.026). Differences in CR rate and OS between patients treated with Flagida, HiDAC and Mitoxantrone + Etoposide were not statistically significant (patients treated palliatively were excluded). **Conclusions.** The EPI is useful for stratification of patients with AML in first relapse into different prognostic groups, which provide important information about probable outcome and for therapeutic decision-making.

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PLASMA CYTOKINE AND ADHESION MOLECULE PROFILE IN PATIENTS TREATED FOR ACUTE MYELOID LEUKEMIA

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Background. Cytokines and adhesion molecules have been studied as markers of immune system activation in various diseases including hematological malignancies. Alterations in this network may have direct effect on the malignant cells or have indirect effect on leukemogenesis through altered functions of bone marrow stromal elements. The knowledge gained from multiple cytokine and adhesion molecule analysis should allow better diagnosis and disease management. **Aims.** The aim of our study was to evaluate plasma cytokine and adhesion molecule profile by biochip array technology in patients treated for acute myeloid leukemia (AML). **Methods.** A total of 15 AML patients (mean age 48.7 ± 12.1 years, median 51, 8 males and 7 females) treated with cyclic chemotherapy (3+7, 2+5, HiDAC) alone or in combination with high-dose chemotherapy (preparative regimen Bu/Cy2 or Cy/TBI) followed by autologous hematopoietic stem cell transplantation were studied. We evaluated plasma levels of the following 22 cytokines and adhesion molecules: interleukins (IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-23), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1), E-Selectin, L-Selectin, P-Selectin, Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox) at the diagnosis of AML (active leukemia) and at 6 months after completion of chemotherapy (durable complete remission /CR/ in all patients). Probability values (p) < 0.01 and lower were considered statistically significant. **Results.** Comparing plasma cytokine and adhesion molecule levels in active leukemia and in durable CR, we found significant increase in plasma IL-7 (5.34 ± 4.32 ng/L vs. 19.62 ± 12.05 ng/L; p < 0.001), EGF (16.48 ± 33.50 ng/L vs. 64.42 ± 35.33 ng/L; p < 0.001) and VEGF (63.93

± 67.85 ng/L vs. 114.39 ± 54.90 ng/L; p < 0.01). On the other hand, we found significant decrease in plasma E-Selectin (30.19 ± 20.46 mcg/L vs. 12.99 ± 8.00 mcg/L; p<0.01). Plasma levels of other evaluated cytokines and adhesion molecules were without significant differences. **Conclusions.** Our results indicate that plasma levels of some cytokines and adhesion molecules (IL-7, EGF, VEGF, E-Selectin) are significantly altered in patients treated for AML, showing activity of the disease. Whether these alterations could serve as a prognostic marker for AML is not known. Further studies in a larger number of patients and comparing cytokine and adhesion molecule levels with established prognostic markers (cytogenetics, molecular genetics) will be needed to define the potential role of these and additional markers in the risk stratification of AML patients.The work was supported by research projects MO 0FVZ0000503 and MZO 00179906.

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FRACTIONATED DOSES OF GEMTUZUMAB OZOGAMICIN COMBINED WITH STANDARD CHEMOTHERAPY AS SALVAGE TREATMENT FOR YOUNGER PATIENTS WITH RELAPSED ACUTE MYELOID LEUKEMIA.

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Background. Patients with relapsed acute myeloid leukemia (AML) usually have a poor outcome. Chemotherapeutic salvage regimens can yield complete remission (CR) in less than 50% of patients, with a 3-year overall survival (OS) rate of less than 30%.Used as single agent therapy for relapsing AML, gemtuzumab ozogamicin (GO) allows 25-35% CR, and some studies combining GO and chemotherapy are showing promising results. **Aims.** Retrospective evaluation of efficacy and toxicity of the association of fractionated doses of GO and standard intensive chemotherapy as salvage treatment for younger patients with relapsed AML. **Methods.** Included patients were aged less than 60 years, with a CD33+ AML in first relapse and an allogeneic transplantation planned in CR2. Salvage regimen consisted of GO 3mg/m^2 intravenously over 2 hours on days 1-4-7, cytarabine 200 mg/m^2 in continuous intravenous infusion over 24 hours on days 1 to 7 and daunorubicine 60mg/m^2 or idarubicine 12mg/m^2 intravenously over 30 minutes on days 1 to 3. **Results.** Fourteen patients were treated between April 2008 and April 2011. There were 6 male and 8 female, median age was 46 years (29-58), and median white blood cell count was 3.4 10^9/L (0.9-19). At diagnosis, according to the European Leukemia Net classification, there were 7, 2, 3 and 2 patients in the favourable, intermediate I, intermediate II and adverse groups, respectively. All the patients have been treated with standard AML practices and had received high or intermediate doses of cytarabine as consolidation therapy. Median CR1 duration was 11 months (1-42), with CR1 duration lesser than one year in 8 patients.Salvage therapy was performed as scheduled for the 14 patients, using daunorubicine in 12 patients and idarubicine in 2 patients. Six CR and 5 CR with delayed platelet recovery (CRp) were observed, allowing a 79% overall response rate. Median times to neutrophil (>0.5 10^9/L) and platelet (>20 10^9/L) recovery were 29 days (23-32) and 36 days (28-48), respectively. Treatment was complicated by three mild and one moderate veno-occlusive disease (VOD).Transplantations were performed in the 11 CR/CRp patients' within a median delay of 4 months (3-10). Ten patients were still in remission whereas one relapsed before transplant and was transplanted with refractory AML. Two mild VOD were observed after transplantation.Median follow-up of all the population is 10 months (2-45), and 6 patients are still alive and in CR with a median follow-up of 25 months (10-45), corresponding to a median overall survival (OS) rate of 10 months and an estimated 2-year OS of 42%. Five of the 11 transplanted patients died within a 5 months (1-10) median post transplant delay, corresponding to an estimated 2-year OS of 53% (median OS non reached). Death causes after transplantation were GVHD in three patients and AML relapse in two patients. **Conclusions.** In this limited size retrospective study on younger patients with AML in first relapse, fractionated doses of GO combined with 3+7 chemotherapy showed high anti leukemic activity and good safety profile, enabling to perform transplantation in most patients.

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COGNITIVE FUNCTIONS AND QUALITY OF LIFE IN ACUTE LEUKEMIA PATIENTS

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Background. Chemotherapy for acute leukemia can have an effect on cognition and quality of life. Cognitive impairment and fatigue have been described in patients with AML/MDS, even before initiation of treatment.**Objective:** To assess cognitive evolution, and to measure both depression and quality of life in adult acute leukemia patients, before and after induction treatment. **Methods.** Current longitudinal-prospective study of adult acute leukemia patients treated with aggressive chemotherapy. Eligible patients are enrolled and are administered a comprehensive cognitive test battery, within five days after admission (T0) and after completion of induction treatment (T1). Cognitive functions assessed are attention, executive functions, motor dexterity, and verbal memory (tested with AVLT, COWA, PPT, SCWT, and TMT). Both depression and quality of life were assessed with two self-report questionnaires (assessed with CES-D and EORTC-QLQ-C30). **Results.** Thirty-five (n=35) adult acute leukemia patients were included between 01/2009 and 12/2011. The median age was 46 years, 53% were female. The median duration of education was 13 years. 88% had AML and 12% had ALL. Baseline mean hematological values were: WBC count (7,2 10E3/uL), RBC count (3,1 10E6/uL), and HgB (9,8 g/dL). Induction treatment used in AML patients was either the AML 2/95 protocol or the HOVON 102 AML protocol, in ALL patients the GMALL 7/03 protocol was used. Baseline cognitive functions were normal in different cognitive domains. Short-term evolution of cognitive functions was observed in executive functions (SCWT IV-III and TMT B), and in verbal learning (AVLT A1-5), both showed a significant improvement (all p<.005). Baseline levels of distress and depression were statistically significant, and adult leukemia patients had problems on all functional scales, except for cognitive function (CF=70), and on the symptom scale fatigue (F). Short-term changes in quality of life were observed in depression, global health score (QoL), functional scale emotional function (EF), and symptom scale fatigue (F) and pain (P). **Conclusions.** At baseline, adult acute leukemia patients had normal cognitive functions, except for verbal learning and especially motor dexterity. Depression and problems on quality of life were observed at baseline, whereas at follow-up only role and social function, and also fatigue were still impaired.

Table 1. Changes in cognition and quality of life (n=25-27/28).

	T0	T1	p
Cognition (n=25/27)			
Executive function			
SCWT IV-II	57	50	**.002**
TMT B	94	80	**.001**
Verbal learning			
AVLT A1-A5	48	53	**.000**
Depression (n=28)	21	15	**.006**
Global health (n=28)	36	58	**.000**
Physical function	62	72	.060
Role function	40	46	.499
Emotional function	58	76	**.000**
Cognitive function	70	78	.206
Social function	58	51	.282
Fatigue	66	46	**.012**
Nausea & Vomiting	26	14	.094
Pain	49	20	**.001**

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WHICH IS MORE IMPORTANT: DURATION OR QUALITY OF LIFE IN PATIENTS WITH LEUKAEMIA AND LYMPHOMA?

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Background. It is usefull to regularly test the patients opinion according to the various issues raised by medical practice. Duration and quality of life are among them. Their responses are influenced by the hospital management and can provide starting points for improving it. **Aims.** We aimed to study the patients opinion from southern Transylvania on the relationship between duration and quality of life of patients with hematologic malignancies.**Material and Methods.** transversal study was performed on a sample of 250 consecutive hospitalized patients, who agreed to respond with 'yes', 'no' or ' don't know' to a questionnaire on the management of life quality of patients with leukemia and malignant lymphoma. The results were analyzed and they allowed conclusions with implications for clinical practice. **Results.** The mean age of patients surveyed was 55.64+/-16.01 years. Distribution by gender: women 44.39%, 55.61% men. About 75.61% of respondents felt that finding the diagnosis of leukemia or lymphoma is traumatic for patients and 16.59% of them said, surprisingly, not. 72.26% of the first measured depression between 8 and 10 notes on a scale of 1-10. 54.63% of them thought that it will be less traumatic for the patient if the doctor would not say the diagnosis of malignant hemopathy, but 69.27% of them considered depression caused by finding the diagnosis preferable to ignoring it for a long time. About half of respondents (49.27%) considered quality of life more important, and the other half - the duration of life of patients with malignant hemopathies. Most responders (72.68%) felt that it is more important how you live and only 25.37% - how much you live. Most (61.95%) agreed with aggressive chemotherapy, and only 31.22% opted for palliative treatment for these patients. Almost half (54.15%) agreed with the recommendation of herbal medicine. Almost all (93.17%) agreed to the idea that the family can contribute to the quality of life of patients. 91.71% felt that psychotherapy can improve the quality of life and 82.93% considered that psychotherapy should be done by the hematologist, in the absence of a psychologist since psychological balance can positively influence the course of disease (87.80% of respondents). 78.05% of them agreed to the idea of correcting anemia with erythropoietins, which could increase the quality of life of patients, even if they have adverse effects (60% of respondents). 77.07% agreed the idea of participation in multicenter studies with new drugs. 96.59% of them, would agree to donate blood for these patients, even if they were Romani (gypsy ethnic group) (95.12%), and even peripheral stem cells for allogeneic transplantation (87.32%).**Summary**: Most people put in the spotlight the quality of life and sustain all therapeutic means that improve it. In this respect they support the benefits of chemotherapy with curative intent and erythropoietin. They are concerned about depression in patients with malignant hemopathies and agree to any measure that might reduce it. They agree, as donors, to participate voluntarily in helping them.

Cytogenetics

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THE NUP98-NSD1 FUSION IN ASSOCIATION WITH FLT3-ITD MUTATION IDENTIFIES A PROGNOSTICALLY RELEVANT SUBGROUP OF PAEDIATRIC AML, WHICH CAN BE SALVAGED BY ALLOGENEIC TRANSPLANT

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Background. Cytogenetics provides the most powerful independent prognostic factor in AML presenting in children and younger adults, providing the framework for risk-stratified treatment approaches. However, approximately half the cases presenting in this age group fall into the intermediate cytogenetic risk category, within which patients have vastly different outcomes. The cytogenetically cryptic t(5;11)(q35;p15) leading to the *NUP98-NSD1* fusion, is a rare but recurrent gene rearrangement, recently reported to identify a previously unrecognised group of young AML patients with a poor prognosis for whom new treatment strategies are needed (Hollink et al, Blood 2011). **Aims.** The aim of this study was to determine the frequency of the *NUP98-NSD1* fusion in a series of 54 unselected *de novo* paediatric AMLs (median age 9yrs, range 0-18yrs) and to develop an RT-qPCR assay to track individual patient response to treatment. **Method.** Screening for the *NUP98-NSD1* fusions was performed using reverse-transcription PCR (RT-PCR) together with FISH confirmation of all positive cases. A reverse transcription-quantitative PCR (RT-qPCR) assay was designed to allow sensitive sequential analysis of post treatment material, where available. The sensitivity routinely achieved was sufficient to detect normalised *NUP98-NSD1* transcripts levels 4 logs below those seen at diagnosis. **Results.** Four positive cases (7%) were identified; three *de novo* AML and one t-AML following chemotherapy for osteosarcoma two years prior to presentation with AML. All were older children (median age 16yrs, range 13-18yrs) with very high white blood counts (WBC) at presentation, without favourable cytogenetic markers. All had a concurrent *FLT3*-itd and all lacked NPM1 and CEBPa mutations.All four patients received an allograft at 3,4,4 and 13 months post diagnosis respectively. One patient died of transplant related complications, but was shown to have low level MRD immediately prior to transplant by retrospective RT-qPCR analysis. Two patients remain alive and in molecular remission more than two years post-diagnosis, despite demonstrating a high tumour burden prior to transplant, suggesting a potential survival benefit from transplant. The fourth case failed to achieve molecular remission for the *NUP98-NSD1* gene fusion at any of the time points measured pre-or post-transplant and died 11 months post diagnosis of metastases from a pre-existing osteosarcoma. **Conclusions.** Our data suggests a non-random association of *NUP98-NSD1* with *FLT3*-itd in a group of older children with high WBC's with a poor prognosis who may benefit from transplant. The incidence of *NUP98-NSD1* rearrangement, potentially as high as 7% of *de novo* paediatric AML, is sufficient to recommend routine screening of *NUP98-NSD1* in combination with *FLT3*-itd for all new diagnostic cases, particularly in the absence of otherwise favourable cytogenetic markers, to accurately determine risk and allow for consideration of early transplant.

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GENE MUTATIONS IN CHILDHOOD ACUTE MYELOID LEUKEMIA WITH SPECIAL REFERENCE ON THE MUTATIONS OF EPIGENETIC REGULATORS INCLUDING ASXL1, IDH1/2, AND DNMT3A

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Background and Purpose. Four genes involving the epigenetic regulators, i.e. *ASXL1, DNMT3A, IDH1* and *IDH2*, have recently been described in adult acute myeloid leukemia (AML) and were associated with poor outcomes. In childhood AML, the reports on these gene mutations have been very rare, especially there has been no report on *ASXL1* gene mutations. In addition, comprehensive analyses of gene mutations in *de novo* childhood AML have been limited. We aimed to determine the genetic alterations in pediatric AML patients with special reference on the mutations of epigenetic regulators. **Materials and Methods.** We analyzed 18 gene mutations in 206 children with *de novo* AML

aged from 1 month to 18 years diagnosed between December 1995 and May 2011. The mutated genes examined included class I gene involving signaling and RAS pathways *(FLT3*-ITD, *FLT3*-TKD, *C-FMS*, *C-KIT, NRAS, KRAS, PTPN11, JAK2*V617F), class II affecting transcription and differentiation (*CEBPα*, *RUNX1*, *MLL*-PTD, and *NPM1*), class III of tumor suppressor genes (*WT-1* and *P53)*, and class IV of epigenetic regulators (*ASXL1*, *DNMT3A*, *IDH1* and *IDH2*). Mutational analysis was performed with PCR-based assays followed by direct sequencing. **Results.** One hundred and twenty of 206 patients (58%) were found to have at least one mutation; 51% had class I, 14% had class II, 6% had class III and 4% had class IV mutations. The most frequent gene mutations were *FLT*3 (21.6%; *FLT3*-ITD 14.9% and *FLT3*-TKD 7.4%), *RAS* (15.6%; *N-RAS* 8.3% and *K-RAS* 7.4%), and *C-KIT* 11.5%. Together, 15.5% had more than one mutation. Of the 7 patients with mutated genes of epigenetic regulators, *ASXL1* mutations were detected in 2 of 175 patients (E635fsX649 and P835fsX841), *DNMT3A* mutations in 2 of 168 (W795S and R882H), *IDH1* mutations in 2 of 177 (R132C and R132H), and *IDH2* mutation in 1 of 177 (R140Q). The 2 patients with *ASXL1* mutations were both of t(8;21), without cooperating mutation with other 17 genes analyzed. In the present series, 4 patients had *MLL*-PTD; two of them, both of FAB M0 subtype, harbored *IDH1* gene mutations compared with none of the 173 patients with non-*MLL*-PTD AML ($P < 0.0001$). One of the two *IDH1* mutated patients had *FLT3*-ITD, the other patient had trisomy 21and *RUNX1* mutation. The only one patient harboring *IDH2* mutation had normal karyotype and also had *FLT3*-ITD and *NPM1* mutations. Of the 2 patients with *DNMT3A* mutations, one cooperated with *PTPN11* and *CEBPα* mutations; the other patient had *MLL* translocation, *FLT3*-TKD and *WT1* mutations. All patients were treated with Taiwan Pediatric Oncology Group-AML 97 protocols. Since the numbers of patients with the 4 gene mutations were very small, it precluded the meaningful analysis of the prognostic impact. **Conclusions.** The present study on 18 gene mutations in a relatively large cohort of children with *de novo* AML in Taiwan showed 58% of patients had at least one gene mutation. The frequencies of mutations of epigenetic regulators were very rare, *ASXL1* mutations were associated with t(8;21) and *IDH1* mutations were significantly associated with *MLL*-PTD. Support by grants NSC-96-2314-B-195-006-MY3 and MMH-E-99009.

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FROM CYTOGENETICS TO MOLECULAR BIOLOGY: MAPPING OF UNIQUE CHROMOSOMAL ABNORMALITIES AT THE NUCLEOTIDE LEVEL

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Background. Acute myeloid leukemias (AML) in adulthood represent a heterogeneous entity, characterized by a recurrent chromosomal/genetic abnormality in only 50% of cases. In the remaining individuals, no common molecular abnormality can be identified using standard diagnostic screening (AcutePlexX[1], mutations in NPM1, WT1, CEBPa and others), though unique cytogenetic abnormalities can often be detected. As many AML patients are eligible for curative treatment, techniques allowing specific and sensitive minimal residual disease (MRD) monitoring are highly needed. **Aims.** To develop a technique that would allow mapping of cytogenetically identified unique clone-specific abnormalities from the chromosome level to the nucleotide level, enabling us to develop clone-specific quantitative Real-Time PCR assays for sensitive and specific MRD monitoring. **Methods.** Molecular-cytogenetic techniques (mFISH, mBAND, BAC-FISH), chromosome microdissection, next generation sequencing, long-range PCR and direct Sanger sequencing were used to map the chromosomal translocation in the der(10)t(3;10)(p21.3;q23) characteristic for the cell line K562. This model cell line was chosen to show the feasibility and reproducibility of the technique as a proof of principle, with prospective continuation to authentic patient samples. After cytogenetic identification of the chromosomal translocation (Figure 1A), the derivative chromosome was microdissected using a fine needle (Figure 1B). The microdissected fragments were directly subjected to whole genome amplification (WGA; Figure 1C) and then sequenced on the GS Junior platform for next generation sequencing (Figure 1D). Obtained reads were aligned to reference sequences of chromosomes 3 and 10, using in-house developed software (Figure 1E). The last mapped reads from both chromosomes were used as docking sites for primers for long-range PCR to amplify the putative breakpoint (Figure 1F). The long-range PCR products were directly sequenced using Sanger sequencing to reveal the precise nucleotide sequence of the breakpoint (Figure 1G). **Results.** Using a combination of cytogenetic and molecular approaches, we mapped the K562-unique translocation in der(10)t(3;10)(p21.3;q23) from the chromosomal level to the nucleotide level (Figure 1). Direct sequencing of this breakpoint revealed a head-to-head fusion of genes CDC25A and GRID1. The time demand of the whole procedure, starting from mFISH and ending with the actual sequence of the breakpoint was approximately 6 weeks. This is optimal timing for a standard clinical setting, when the laboratory receives the first follow-up samples one month after diagnosis. **Summary and Conclusions.** Current technologies of molecular cytogenetics and molecular biology open new vistas in the detection and identification of unique, clone-specific genetic abnormalities in patients with AML. As only half of the individuals with AML can be molecularly MRD followed-up using recurrent genetic abnormalities, there is still a sizeable proportion of patients who might benefit from the identification of the "finger prints"of their malignant cells for the design of clone-specific MRD Real-Time PCR assays. Our work clearly shows that "walking"from the chromosomal level to the nucleotide level is feasible and readily applicable for eligible AML patients. **Acknowledgements.** The work was supported by the Grant Agency of Ministry of Industry and Trade of the Czech Republic and in part by the Monika-Kutzner Foundation.

Reference

Plachy R. et al., 2011; Blood, 118:1083, Abstract 2526.

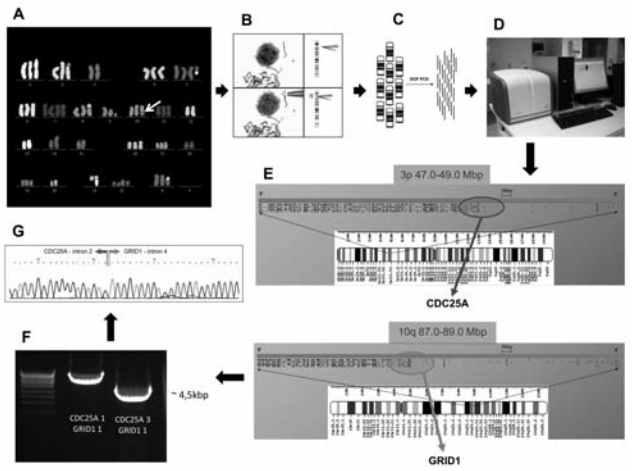

Figure 1. The outline of the technique used to walk from the chromosome level to the nucleotide level.

0685

RUNX1 DISRUPTED BY A COMPLEX REARRANGEMENT OF CHROMOSOME 21 CAUSES FAMILIAL PREDISPOSITION TO HEMATOLOGICAL MALIGNANCIES

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Background. Clinicians are increasingly aware of familial predisposition to myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML). In a substantial part of these families there is a mutation in the RUNX1 gene on chromosome 21q22. Chromosomal rearrangements, like deletions, involving the RUNX1 locus are less frequently described. *Aims and* **Methods.** In our hospital, a male patient (age 28) presented with mild thrombocytopenia and abnormal platelet aggregation. Family history revealed five relatives with leukemia (3 AML, 1 ALL and 1 of unknown origin). Our patient and his mother were not affected with leukemia. To elucidate the underlying genetic aberration in this family, blood and bone marrow samples of the index patient and his mother were examined by conventional karyotyping, fluorescence in situ hybridization (FISH), Agilent 180k microarray (AMADID 023363), Nimblegen capture array (custom made) and mutation analysis. **Results.** Although karyotyping and mutation analysis of both the index patient and his mother showed normal results, interphase FISH revealed 3 signals for the RUNX1 locus. Array CGH demonstrated 3 minor aberrations (2 gains and 1 loss) of distinct regions of chromosome 21q22, but could not explain the extra FISH signal. Therefore, it was speculated that an intra-chromosomal rearrangement with a breakpoint through RUNX1 had resulted in a split signal. This was confirmed by capture array data, which demonstrated a fusion of the 5' RUNX1 breakpoint to a chromosome 21 sequence 11 Mb upstream (same orientation). **Summary and Conclusions.** Based on the results so far, it was concluded that a complex rearrangement with several breakpoints on chromosome 21 resulted in the disruption of the RUNX1 gene. Currently, further investigations by using a novel next-generation sequencing technique are ongoing. We will perform genome-wide long mate-pair sequencing in order to delineate an exact map of the affect-

ed chromosome 21 in this family. By using this map, a diagnostic test for carriership analysis will be made available for screening family members at risk of developing MDS/AML themselves or as potential transplantation donors. Affected family members, especially the patient diagnosed with ALL, will also be examined. It is important to realize that adequate screening of families for predisposition to MDS/AML not only involves mutation analysis, but that other methods (e.g. aCGH, FISH) and genetic counseling should also be considered.

0686

HIGH RESOLUTION MICROARRAY-BASED GENOMIC PROFILING FOR THE IDENTIFICATION OF SMALL GENETIC LESIONS AND SUBSEQUENT DESIGN OF MINIMAL RESIDUAL DISEASE TARGETS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. In acute lymphoblastic leukemia (ALL) specific genomic abnormalities provide important clinical information. We previously demonstrated that microarray-based genomic profiling allows the detection of focal genomic losses, frequently harboring clinically relevant ALL-related genes, such as *EBF1*, *CDKN2A/B*, *PAX5*, *ETV6*, *BTG1* and *IKZF1*. The latter gene was recently shown to be associated with a high relapse rate in patients with ALL (Kuiper et al., 2010, Leukemia). The ability to detect focal lesions, however, largely depends on the resolution of the platform used.The analysis of minimal residual disease (MRD) at early time points during therapy has been widely implemented as an accurate prognostic parameter to determine treatment strategy. MRD targets usually include immunoglobulin and/or T-cell receptor rearrangements detected in the major clone at diagnosis. Accurate array-based mapping of recurrent abnormalities on ALL may facilitate the development of ALL-specific MRD targets, as we recently described for *IKZF1* (Venn et al., 2012 Leukemia). **Aims.** We evaluated the performance of a novel high resolution genome-wide microarray platform (Cytoscan HD, Affymetrix) for the detection of ALL-specific genomic abnormalities. Due to the high coverage of probes on this platform, we also evaluated its suitability for accurate identification of breakpoints covering deletions of leukemia-specific genes, which could subsequently serve as markers in sensitive PCR-based MRD tests. **Methods.** The Cytocan HD platform (2.7 million probes) was used for genomic profiling of 8 patients with ALL. Genomic lesions affecting recurrent gene loci (see above) were evaluated and the positions of the breakpoints were accurately mapped. To confirm the focal genomic losses identified by the CytoScan HD, the samples were also evaluated with a commercially available multiplex ligation-dependent probe amplification (MLPA) test (MRC Holland) and breakpoint-spanning PCR. **Results.** In 8 ALL-patients tested we identified 21 deletions (sizes down to 10 kb) encompassing genes commonly affected in ALL, which were all validated by MLPA. Eleven of these deletions were smaller than 100 kb, which is below the detection level of most commonly used micro-array platforms. Next, we analyzed the resolution with which the breakpoints in the 21 deletions could be mapped by using the Cytoscan HD microarray data. In 8 of the 21 deletions covering leukemia genes both breakpoints were located in a region less than 1 kb. These data can be very helpful in designing leukemia-specific PCR primer sets in individual patients. By applying this approach we were able to identify such primer sets in 7 of the 8 patients and for which 5 deletion-specific PCR products were validated, so far. This demonstrates the additive value of the Cytoscan HD platform in designing PCR-based targets which can be used for MRD analysis. **Summary and Conclusions.** We conclude that the Cytoscan HD microarray platform has improved detection rate of small focal genomic losses, including *IKZF1*, as compared to other platforms. Additionally, we demonstrated the power of this platform for accurately identifying breakpoints of genomic losses for development of patient-specific targets for MRD analysis.

0687

NUCLEOPORIN GENES INVOLVEMENT IN HAEMATOPOIETIC DISORDERS

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Background. Nucleoporins (NUPs), a group of ~30 proteins, constitute the Nuclear Pore Complex, a large multi-protein channel which perforates the nuclear envelope and mediates macromolecular import and export between nucleus and cytoplasm. *NUP98*/11p15 and *NUP214*/9q34 are involved in the pathogenesis of haematopoietic disorders. In chromosomal translocations or inversions with different partner genes both produce abnormal fusion proteins with oncogenic properties. Although rearrangements are frequently cryptic, conventional cytogenetics may be predictive of *NUP98* involvement in around 20-30% of cases of Acute Myeloid Leukemias/Myelodysplastyc Syndromes with an 11p15 chromosome change. NUP98 maintains its 5'-end in all fusions while partners always contribute with the 3'-end. **Aims.** To investigate the involvement of 30 NUP genes in patients with diverse haematological disorders and abnormal karyotypes involving chromosomal regions/arms containing one or more NUP genes. **Methods.** The study included 194 cases (145 myeloid and 49 lymphoid neoplasms) collected from files of the Laboratory of Cytogenetics and Molecular Genetics of the University of Perugia (Prof. Cristina Mecucci). An additional series of 25 cases with myeloid neoplasms with 11p15 changes was provided by Prof. Christine Harrison (Leukemia Research Cytogenetics Group, Northern Institute for Cancer Research, Newcastle University). Genomic clones were selected to test each NUP with a double colour break apart test. The following NUPs were investigated: *TMEM48*/1p32, 25 cases; *TPR*/1q31, 21 cases; *NUP133*/1q42, 20 cases; *NUP358*/2q13, 16 cases ; *NUP35*/2q32, 17 cases; *NUP210*/3p25, 14 cases; *SEC13*/3p25 14 cases*; NUP54/4q21,* 3 cases*; NUP155*/5p13 3 cases; *NUP153*/6p22, 13 cases; *NUP43*/6q24, 23 cases; *NUPL2*/7p22, 18 cases; *POM121*/7q11.2 18 cases; *NUP205*/7q33 22 cases; *GLE1*/9q34, 11 cases; *NUP188*/9q34, RP11-98H23 (5'), RP11-167N5 (3') 11 cases; *NUP214*/9q34, 11 cases; *NUP160*/11p11.5, RP11-346F1 (5'), RP11-112L21 (3') 10 cases; *NUP98*/11p15.5 35 cases; *AAAS*/12q13, 7 cases; *NUP107*/12q15 8 cases; *NUP37*/12q23 8 cases; *NUPL1*/13q12, 13 cases; *NUP93*/16q13, 4 cases; *NUP88*/17p13, 27 cases; *NUP85*/17q25, 9 cases; *SEH1L*/18p11.2 9 cases; *NUP62*/19q13.33, 1 case; *RAE1*/20q13 11 cases; *NUP50*/22q13, 10 cases. **Results.** *NUP98* was the only NUP gene shown to be directly involved in structural chromosomal rearrangements. Other NUPs underwent deletions or gains, most of them as expected were according to chromosome losses or gains as described in the karyotypes. Our *NUP98* break apart FISH test showed 5/35 (14%) positive cases among myeloid malignancies with a breakpoint at 11p (between p11 and pter). Additional FISH studies confirmed a *NUP98* fusion and characterized the partner gene as: *HOXA*/7p15 in 2 cases, *NSD1*/5q35.1 and *HOXC*/12q13, one case each. In the fifth case the *NUP98* 3' probe was deleted, suggesting rearrangement/fusion of the retained 5'-end. Unfortunately the study could not be finalized due to lack of biologic material. **Summary and Conclusions.** According to our results *NUP98* in karyotypes from myeloid malignancies with visible 11p15 abnormalities was the only NUP gene found to be directly involved in chromosomal rearrangements. Therefore, due to multiple known fusion partners of *NUP98*, FISH screening is recommended for precise diagnosis of these patients and for fusion partners characterization.

0688

DETECTION OF LYMPHOBLASTIC TRANSFORMATION IN CHRONIC MYELOID LEUKEMIA AND REVEALING THE CLONAL ORIGIN OF RELAPSE IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA USING MLPA

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Background. Multiplex ligation-dependent probe amplification (MLPA) is a robust technique to simultaneously detect copy number abnormalities (CNAs) at several loci. CNA profile provides valuable information for risk assessment of various hematological malignancies. **Aims.** We used MLPA to stratify samples from CML patients in chronic phase (CP) or in lymphoblastic transformation (lyBC) and to discriminate CML-CP cases responding or resistant to imatinib therapy. Furthermore, a large number of prognostically relevant CNAs were screened in pediatric acute lymphoblastic leukemia (pALL) using matched samples of diagnosis and relapse. **Methods.** We investigated bone marrow samples from 30 patients with CML-CP without evidence of blastic crisis (15 responder and 15 resistant to imatinib therapy), from 9 patients with CML undergone lymphoblastic transformation and from 49 patients diagnosed with pALL. Ethical Committee approval and written informed consent have been obtained for the study. Two different MLPA kits were applied allowing to detect gene locus centered CNAs at 58 different loci. Tumor cell ratio measured by flow cytometry and cytogenetic data were also considered at the calculation and

interpretation of copy number gains and losses. **Results.** CNAs were detected in a low proportion (2/30) of patients with CML-CP thus subclassification of cases being resistant or responding to imatinib therapy proved to be impossible. Investigating patients with CML having lyBC at diagnosis or at any follow-up time points, we did not find CNAs in their samples acquired during CP; however, in all but one samples withdrawn during lyBC, we observed several CNAs; an average of 22 probes per sample showed abnormal patterns. *IKZF1* was the most frequently affected gene followed by *PAX5*, *CDKN2*, *MIR31*, *MIR24-2*, *ETV6* and *EBF1*. CNAs were detected in 73% of patients with pALL; *CDKN* was the most frequently affected gene followed by *ETV6*, *PAX5*, *MIR31*, *IKZF1*, *RB1*, *BTG1*, *EBF1*, *IKZF3* and *MIR23A*. Comparing the CNA profile of diagnostic and relapsed samples of patients with pALL, in one-third of cases we found patterns referring to clonal evolution process (i.e. cells harbouring both original and newly acquired abnormalities recurred). In two-thirds of cases, cell populations dominating at different time points were not clonally related but independently arisen suggesting the presence of a preleukemic cell pool. **Conclusions.** MLPA has been used for investigating patients with CML for the first time and it proved to be an efficient tool for detecting transition not only from chronic phase to lymphoblastic crisis but vice versa as well. Evaluating samples from children with ALL, combined application of various MLPA probe mixes allowed us to detect a panel of CNAs broader than ever before. Identification of the origin of the malignant clone at relapse will have great impact on future treatment strategies in pALL. In this study, if the original leukemic clone recurred at relapse the patient died within a short period of time while the occurrence of a clonally independent cell population (i.e. a 'new disease') was associated with optimal response to the administered ALL-REZ BFM 2002 protocol.

0689

A CONTRIBUTION TO THE CYTOGENETIC INVESTIGATION OF MONOCLONAL B-CELL LYMPHOCYTOSIS

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Background. Monoclonal B-cell lymphocytosis (MBL) has recently attracted a significant research interest as the prelude of chronic lymphoproliferations. However, despite the advances and wide application of flow cytometry which allow for the identification of the disorder in an increasing number of patients, the cytogenetic investigation of MBL remains a technical challenge and the relevant data is scarce. **Aims.** In this study, we have applied interphase FISH (i-FISH) for the investigation of chromosomal aberration frequently found in chronic lymphoproliferations in patients with documented MBL. **Methods.** 39 men and 21 women with MBL, according to the currenty established diagnostic criteria, were included in this study. Flow cytometry and i-FISH were performed on a peripheral blood sample. In cases with a B-cell count <15% of WBC, the i-FISH study was performed on purified B-cells after immunomagnetic separation targeting CD19. The presence of 13q-,+12, -11/11q-, 17/17p-,-6/6q- , t(11;14) and rearrangement of the BCL2 and IGH genes, was investigated in all cases, regardless of the morphological features or the phenotype. **Results.** High-sensitivity flow cytometry showed a chronic lymphocytic leukemia (CLL)-like (CD5+CD23+), a mantle cell lymphoma (MCL)-like (CD5+CD23-) and an "atypical"phenotype in 44, 5, and 11 cases, respectively. i-FISH was positive for t(11;14) in 3 cases (all with a MCL phenotype), +12 in 3 cases (all with CLL phenotype) and 13q-, involving 13q14, in 23 cases (19 with CLL and 3 with "atypical"phenotype. In one case, +12 was concurrent with hemizygous 13q-. Of the rest 22 cases with 13q-, the deletion was hemizygous in 16 (15 of which with CD5+CD23+, 1 with CD5-CD23- B-cell population), homozygous in 4 (all CD5+CD23+) and hemizygous coexisting with homozygous in 2 cases (CD5+CD23+). During the follow-up period (at least 24 months), evolution into "clinical"CLL (RAI stage 0) was observed in only one case (CD5+CD23+, with 13q deletion and no other aberrations upon diagnosis of the CLL). **Conclusions.** i. The most frequent finding in this series of MBL cases was 13q14 deletion. It was observed mainly - but not exclusively - in CD5+CD23+ phenotype, at a rate comparable to that in clinical CLL. On the other hand, the absence of aberrations denoting an adverse prognosis in overt lymphoproliferations - such as deletion of the ATM or p53 genes - implies that CD5+CD23+ MBL correspond to the low risk CLL. ii. Evidence for clonal evolution (namely homozygous and concurrent hemizygous/homozygous 13q14 deletion or the coexistence of 13q14 deletion with +12) indicates genetic instability in MBL already at the preclinical stage, but the biological and clinical significance of this phenomenon requires further investigation. iii. The presence of t(11;14) among cases with consistent phenotype is perhaps equivalent to the diagnosis of the recently described "indolent"MCL. The follow-up of these cases at the clinical and genetic level may provide important clues on the mechanisms of oncogenesis in human lymphomas.

0690

FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN ADULTS

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Background. Familial Hemophagocytic lymphohistiocytosis (FHL) is a rare immune deficiency with defective cytotoxic function. The age at onset is usually young and the natural course is rapidly fatal if untreated. A later onset of the disease has been sporadically reported even in adolescents and adults. **Aims.** To report all patients diagnosed with FHL at an age of 18 year or older and enrolled in the Italian Registry of HLH. **Methods.** A retrospective data collection was performed. FHL was defined according to the diagnostic criteria established by the Histiocyte Society. Patient information were collected on specific forms. Biological samples were collected for immunological studies (flowcytometry analysis of perforin expression and degranulation assay, cellular cytotoxicity) and direct sequencing of currently known FHL-related genes. Data were pooled in a common database and analysed. **Results.** A total of nine patients were diagnosed with FHL based on the finding of biallelic mutations in one FHL-related gene. They included 6 males and 3 females, from eight unrelated families; their age ranged between 18 years and 43 years (median, 25.1 years). Seven families were of Italian origin and one was from Colombia. Family history was unremarkable in six families at the time of the diagnosis. Their genetic diagnoses were: FHL2 (n=6), FHL3 (n=1), FHL5 (n=1), XLP1 (n=1). Isolated asymptomatic hypertransaminasemia followed by neurologic symptoms, lymphoproliferative-like manifestations, fulminant mononucleosis, mono-like episodes were the first manifestations of the disease in four patients; a clinical onset with full-blown rapidly progressive FHL was detected in only three patients. Outcome, molecular and functional data are reported in the Table 1.

Table 1.

UPN	Gender/ Age (years)	Diagnosis	Gene/ Protein	Genotype	Fever	Splenome galy	Platelet (count/mm³)	Fibrinogen (mg/dl)	Ferritin (ng/ml)	Hemophagocytosis	Functional study	Course and outcome
198	M/18	FHL3	UNC13D/ Munc13-4	c.1847A>G p. E615G c.1847A>G p. E615G	+	+	25	90	NP	+	Absent NK cell cytotoxicity	Response to initial therapy according to HLH94, dead of septicemia at 18.8 years
199	M/18	XLP1	SH2D1A/ SAP	Hemizygous del Exon1	+	+	19	120	NP	+	ND	Early death of progressive disease. Diagnosis confirmed after death
232	F/27	FHL2	PRF1/ Perforin	c.272C>T p. A91V c.1122G>A p.W374X	+	+	25	<100	>10.000	+	Absent perforin expression and NK cell cytotoxicity	Refused allogeneic SCT, dead of progressive disease at 36 yrs
233	M/22	FHL2	PRF1/ Perforin	c.272C>T p. A91V c.1122G>A p.W374X	+	+	20	<100	>10.000	-	Absent perforin expression and NK cell cytotoxicity	Progressive diseases at 37 years, awaiting for SCT
276	M/22	FHL2	PRF1/ Perforin	c.160C>T p. R54C c.272C>T p. A91V	+	+	25	NP	NP	+	ND	Refused SCT, early death of progressive disease
525	M/23	FHL5	STXBP2/ Munc18-2	c.416C>G p.P139R* c.1247-1 G>C p.SPLICE error	+	+	19	120	13.640	-	Defective protein, degranulation and NK cell cytotoxicity	Response to initial therapy according to HLH2004, cured after SCT
557	M/39	FHL2	PRF1/ Perforin	c.452A>T p.H151L* c.452A>T p.H151L*	+	+	30	<100	NP	-	ND	Dead of progressive disease during initial therapy according to HLH2004
577	F/28	FHL2	PRF1/ Perforin	c.695G>A p.R232H c.673C>T p. R225W	+	+	9	69	295	+	ND	Early death of progressive disease
667	M/43	FHL2	PRF1/ Perforin	c.695G>A p.R232H c.1099T>C p.Y367H* c.272C>T p. A91V	+	+	35	440	6.113	+	Absent perforin expression	Initial response to therapy according to HLH2004, dead of early reactivation before SCT

Conclusions. the concept that later onset of FHL is possible has been repeatedly brought to the attention of adult haematologists.Nevertheless adult patients are at most considered as potentially affected by the so-called "secondary"form of HLH rather than by genetic FHL with several implications.These data confirm that FHL may present beyond the paediatric age. The clinical criteria may be not completely fulfilled and unusual or not specific manifestations are common. Based on our experience we suggest adult specialists to consider FHL in the differential diagnosis of patients with cytopenia and liver or central nervous system disorders, especially when a clonal lymphoproliferative disease is sus-

pected but not confirmed. Anyway all patients with fever, splenomegaly, thrombocytopenia and high levels of ferritin, in whom bone marrow aspirate does not document leukemia, should be rapidly screened on peripheral blood lymphocytes by flow-cytometry for the expression of perforin and the ability to degranulate. The combination of these two assays, widely accessible to many haematology or immunology laboratories, can predict the presence of mutations in the *PRF1* or in the UNC13D, STX11, STXBP2 genes providing a robust support to the diagnosis and justification for high-risk chemo-immunotherapy including indication to allogeneic SCT. The question about indication to SCT for patients with milder clinical picture or even in a pre-symptomatic phase remains open.

0691

HIGHLY SENSITIVE MONITORING OF MINIMAL RESIDUAL DISEASE IN FLT3-ITD-POSITIVE ACUTE MYELOID LEUKEMIA PATIENTS WITH A MUTATION SPECIFIC QUANTITATIVE PCR METHOD

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Background. Minimal residual disease (MRD) monitoring in patients with acute myeloid leukemia (AML) can predict relapse clearly in advance and therefore allows early therapeutic intervention. Moreover, recent studies have highlighted the significance of personalized treatment on the basis of MRD status for improving outcome in AML. The *FLT3* internal tandem duplication (*FLT3*-ITD) occurs in 13-35% of all AML. Clinically, *FLT3*-ITDs have been strongly associated with poor outcome. However, due to the high sequence variability of individual *FLT3*-ITD commonly a universal PCR approach is applied which has a low sensitivity (approx. 1: $5x10^2$). **Aims** The aim of this study was to investigate individual *FLT3*-ITD in detail with respect to prognosis. As well as, to develop a novel cDNA-based, highly sensitive quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) assays for the detection of the *FLT3*-ITD mutation level. **Methods.** We investigated *FLT3*-ITD length and position in 39 AML cases. On the basis of individual *FLT3*-ITD, mutation-specific forward or reverse primers were designed. The expression of *FLT3*-ITD was determined using complementary DNA samples at different points in time diagnosis and subsequent treatment. **Results.** From a total of 409 newly diagnosed AML patients 54 (13%) were *FLT3*-ITD positive. Retrospectively we analyzed ITD mutation of *FLT3* in 39 available cases. Eleven patients had extra insertions of 2-38 base pairs between two repeats. The length of ITD ranged from 3 to 144 base pairs (median 45). Within our cohort patients with *FLT3*-ITD ≥45 base pairs had significantly higher relapse rates (*P*=0.03) and a worse overall survival (*P*=0.03). For the *FLT3*-ITD quantification we developed patient-specific qRT-PCR for 31 individuals with mutation-specific forward or reverse primers. Our method could be applied to 97% of *FLT3*-ITD positive patients and yielded similar results when compared to other high sensitive assays for molecular markers like *MLL*-PTD, *NPM1* mutations or *PML-RARA* (correlation: r=0.99; 0.99 and 0.63, respectively). In one cases a co-amplification of the wild-type could not be avoided resulting in lower sensitivity ($1:10^3$). MRD negativity predicted lasting remission independent of allo-SCT (*N*=7) or non-allo-SCT (*N*=9). All paired diagnostic/relapsed samples showed *FLT3*-ITD positivity. Compared with bone marrow samples *FLT3*-ITD analyses appeared to be equivalently sensitive in peripheral blood. **Conclusions.** We conclude that highly sensitive detection of individual *FLT3*-ITD posses equal prognostic power in AML like established molecular MRD markers. Using this approach MRD guided treatment decisions appear to be justified and should be incorporated in future studies.

0692

CLONAL EVOLUTION OF PROGNOSTICALLY ADVERSE MOLECULAR AND CYTOGENETIC FEATURES DURING THE DISEASE COURSE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. It has been shown that chronic lymphocytic leukemia (CLL) patients displaying a mutation within the tumor suppressor gene *TP53* or a chromosomal deletion 17p (del(17p)) have an adverse prognosis when compared to CLL patients who do not exhibit these anomalies. Furthermore, there is growing evidence that complex chromosomal aberrations, and especially transclocations detected in the neoplastic clone are associated with a similar inferior outcome even if the patients otherwise exhibit prognostically favourable factors. **Aims.** The aim of this study was to investigate clonal evolution in CLL patients with prognostically adverse molecular and cytogenetic features during the course of the disease. **Methods.** Cytogenetic analysis and molecular genetic analysis of *TP53* and immunoglobulin heavy chain variable (IgHV) mutational status were applied to a cohort of 100 CLL patients. **Results.** In our cohort, we identified a total of 23 patients (23%) with progressive disease. Of these, 8 patients showing either a *TP53* mutation and a del(17p) (35%) or a sole *TP53* mutation (n=7, 30%) or a sole del(17p) (n=1, 4%) or complex chromosomal abnormalities (≥ three structural or numerical anomalies) without del(17p) (n=7, 30%). Eighteen patients of the population with progressive CLL (78%) exhibited an unmutated IgHV. In 6 cases, the observed lesion could not be detected in a previous investigation. Out of these cases in 3 patients adel(17p) evolved together with a *TP53* mutation, in one case a *TP53* mutation evolved without a del(17p) and in two cases complex chromosomal aberrations developed. The mean duration for the development of adverse parameters was 21 months. In most cases (n=4) those occurred after treatments with either fludarabine and/or cyclophosphamide and/or rituximab or bendamustine and/or rituximab. However, in two patients clonal evolution was detectable without any causative therapy for CLL. **Summary and Conclusions.** We conclude that clonal evolution of prognostically adverse molecular or cytogenetic lesions can occur in patients with initially favourable risk profile. It appears that this helds true not only for established parameters such as del(17p) and *TP53* mutations but also for newer prognostic factors such as a complex karyotype. Biologically, an overall genetic instability may account for this phenomenon as well as a mitogenic or mutagenic effect caused by cytostatic drugs. It can therefore be discussed, whether assessment of adverse risk factors including conventional karyotyping should be generally performed at an earlier point of the disease course and might be repeated if clinical signs of progression are evident.

0693

REAL-TIME MONITORING OF THE JAK2V617F MUTATION BY ULTRA-RAPID, HIGH SENSITIVE FLUORESCENT AS-LAMP

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Background. The molecular detection of the JAK2V617F mutation is part of the diagnostic work-up of Chronic Myeloproliferative Neoplasms (MPNs), as recommended by the 2008 WHO classification criteria. We recently described an innovative, non PCR method based on an Allele Specific-Loop mediated isothermal AMPlification (AS-LAMP) that specifically amplifies JAK2V617F mutated DNA with a high sensitivity under isothermal conditions (Minnucci G et al Haematologica 2012, *Epub ahead of print*). **Aims.** To improve the reliability of the JAK2V617F detection by implementing an internal control reaction to the AS-LAMP and to allow Real-Time monitoring.

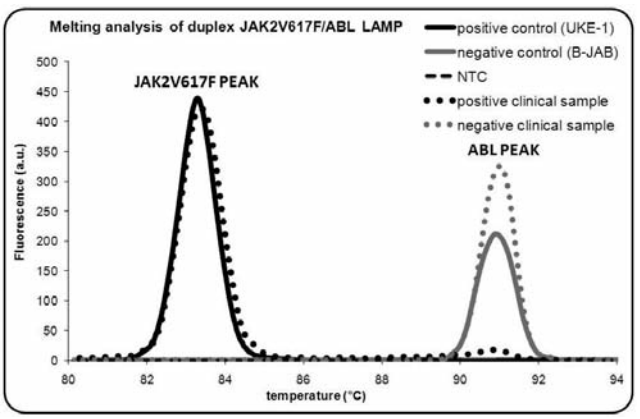

Figure 1. Melting analysis of duplex JAK2V617F/ABL LAMP.

Methods. The isothermal AS-LAMP reaction has been further optimized by the addition of an intercalating dye (Yo-Pro-1, Invitrogen) for fluorescent Real-Time monitoring. Moreover, an internal control reaction has been implemented for simultaneous amplification of the endogenous Abelson gene (ABL) DNA in the same tube. Target and internal control amplified products are characterized by different melting temperatures and can be therefore distinguished by a melting analysis. **Results.** The duplex JAK2V617F/ABL AS-LAMP assay detects in only 30 minutes the JAK2V617F mutation down to 0.05% (mutant DNA from UKE-1 into wild type DNA from B-JAB, 25 ng total). The analytical specificity is 100%, since 315 DNA replicates from wild type cell lines resulted JAK2V617F

negative. This novel JAK2V617F/ABL AS-LAMP assay was validated on DNA extracted from granulocytes of patients affected by PV (n=6), ET (n=7), other hematological malignancies (n=7) and demonstrated 100% concordance with conventional Allele Specific PCR (ASO-PCR). Furthermore, the high sensitivity level of the assay allowed to correctly detect the JAK2V617F mutation on 17 DNA samples extracted directly from peripheral blood, avoiding the step of granulocytes isolation. Interestingly, a direct relationship between the amount of mutant target tested and the height of peaks obtained after melting analysis has been consistently observed. Moreover, all the negative controls, (ET=5), (MF-Pos-ET=3), (healthy donors=10), proved negative for JAK2V617F mutation but showed a correct amplification of the ABL gene. This feature allows to ensure that the absence of JAK2V617F amplification does not depend on a low quality DNA or the presence of wrong reaction conditions or inhibitors.*Conclusions* This Real-Time AS-LAMP assay is a new robust and rapid test to detect the JAK2V617F mutation that can lead to a further development of a fully quantitative assay.

0694

DOUBLE HIT B-CELL LYMPHOMAS

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Background. Double-hit lymphomas are defined by a chromosomal breakpoint affecting C-MYC/8q24 locus (cytogenetic marker of Burkitt lymphoma) in combination with another recurrent breakpoint such as BCL2/18q21, BCL6/3q27 and CCND1/11q13. These tumors are known to behave aggressively with poor prognosis.AIM. In order to evaluate the cytogenetic, morphologic and immunologic peculiarities of these cases, clinical course of the disease and response to treatment we analyzed retrospective data in our cytogenetic database. **Patients and Methods.** For 12-years period in the National Center for Hematology double hits were revealed in 13 cases of B-cell lymphomas, 8 woman and 5 men, mean age 59 years (37-79). In 10 patient conventional cytogenetic analysis and FISH were performed, in 3 cases only paraffin-embedded tissues were available for FISH-assay. FISH studies were performed using a commercially available probes: LSI C-MYC/IGH, cep 8 DF TP, LSI C-MYC BAP, LSI BCL-2/IGH DF TP, LSI BCL-2 BAP, LSI BCL-6 BAP, LSI CCND1/IGH DF TP and LSI CCND1 BAP (Abbott), XL IGL BAProbe and XL IGK BAP (MetaSystems).

Table 1. Characteristics of 13 patients with Double-hit lymphomas.

Pt	Sex/ Age	Diagnosis	Karyotype	FISH	Time of C-MYC+ revelation	Survival, month
1	45/F	ALL	47,XX,der(4)t(1;4)(q31;q33),t(8;14)(q24;q32),+11,der(14)t(8;14)(q24;q32), t(11;22)(q21;q11) [20]	BCL2+, IGL+, t(8;14)+	Initial	7, DOD
2	79/F	FL/ALL	47,XX,der(1)dup(1p),t(8;?)(q24;?),del(9)(p21),+18,t(14;18)(q32;q21), der(22)t(1;22)(p10;q11)[16]/46,XX [1]	t(14;18)+, C-MYC+ t(8;14)-	Relapse	2, DOD
3	63/M	FL	47,XY,der(1)t(1;4)t(1;22)(q24;q11),del(11)(p12),t(14;18)(q32;q21), +der(18)t(14;18)(q32;q21),der(22)add(22)(p10) [20]	t(14;18)+, C-MYC+, t(8;14)-, IGL+	Initial	12, DOD
4	37/M	DLBCL	47,XY,+der(X),del(X)(p11),der(4),del(7)(p13),add(8)(q24),ins(12;?)(13;?),t(14;18)(q32;q21) [6]	t(14;18)+, C-MYC+, t(8;14)-, IGL-, IGK-	Initial	4, DOD
5	72/F	DLBCL	89,XXX,del(X)(q21)x2,-1,-2,-5,-9,-7,der(8)t(8;14) (q24;q32),der(9),-9,-10,-10,+11, t(14;18) (q32;q21)x2,+15,+15,-16,-17,-17,+der(18)t(14;18)(q32;q21)x2,+19,+19,-20, -20,+21,+2mar [10]	t(14;18)+, t(8;14)+	Initial	1, Dearth in induction
6	43/F	DLBLL	-	t(14;18)+, C-MYC+, t(8;14)-, IGL-, IGK-	Initial	1, DOD
7	50/F	DLBCL	46-47,XX,t(2;8)(p21;q24),t(2;14)(q21;q32),+3,t(3;?)(q27;?),del(3)(p21),-6, del(6)(q10),add(13)(q33),der(17),del(18)(?p11),add(22)(q13),+1-2 mar [20]	t(14;18)+, C-MYC+, t(8;14)-, IgK+	Initial	8, DOD
8	78/F	DLBCL	-	BCL6+, C-MYC+, t(8;14)-, IGL-, IGK-	Initial	13+
9	49/F	DLBCL	46,X,der(X)t(7X;?10)(?p11;?p11),del(6)(q21),der(12)dup(12)(q13q24),add(14)(q32), der(21)t(21;22)(q21;q11) [18]	BCL6+, C-MYC+, t(8;14)-, IGL-, IGK-	Progression	2, DOD
10	52/M	DLBCL	-	t(14;18)+, C-MYC+, t(8;14)-, IGL-, IGK-	Initial	6+
11	75/M	MCL	44-48,X,-Y,-1,der(1),del(6)(q15),del(8)(q21),add(8)(q24),-9,der(9)(p21), t(11;14)(q13;q32), -16,-20,-21,+4 mar [cp9]	t(11;14)+, C-MYC+, t(8;14)-, IGL-, IGK+	Relapse	9, DOD
12	65/F	MGUS/PCL	38,X,-X,t(1;2)(q12;p16),del(1)(q12),del(2)(p21),del(6)(q22),add(8)(q24),add(11)(p),t(11;14)(q13;q32), -12,-13,add(14)(q32),der(17)t(12;17)(p21;q23),der(17)t(?;17)(q11;p13), add(21q), add(22) [cp17]	t(11;14)+, C-MYC+, t(8;14)-, IGL-, IGK-	Progression	3, DOD
13	58/M	MM/ Extramedullar plasmacytoma	48,XY,+der(1)del(1)(p32),t(8;11)(q24;q13),t(15;21)(q21;q21),+mar [20]	t(11;14)+, C-MYC+, t(8;14)+	Progression	7, DOD

Results. 7 primary cases were diagnosed as DLBCL in 6 pts, FL in 1 and ALL in 1. Secondary C-MYC rearrangements were observed in 2 relapsed patients, 1 with FL and 1 MCL, after 9 years and 8 months of response, respectively. Two patients with MGUS and MM progressed to C-MYC+ plasmablastic lymphoma after 2 and 5 years after initial diagnosis. In one patient with DLBCL C-MYC rearrangement was not revealed by FISH at the diagnosis and was detected after 8 months in the disease progression. In 7 cases C-MYC translocations were found in coexistence with BCL-2 rearrangements (ALL - 2, FL - 1, DLBCL - 4), in 3 DLBCL patients - with BCL-6, in 3 patients (MCL -1, MM -2) with CCND1 translocations. The most common types of C-MYC alterations were translocations with non-Ig-partners (7 patients), translocations with IGH in 3 cases including one complex translocation t(8;11;14)(q24;q13;q32) and translocations with IGL in 3 patients. Double hits involving two IGH loci were observed in one untreated DLBCL patient within tetraploid clone. High proliferation rate (Ki-67~100%) was observed in all cases except the patient with MCL (~40%). Morphological and immunological features of 9 cases were those of Burkitt lymphoma. All relapsed and progressive patients have died of disease with a median survival after C-MYC revelation of 4,5 months. Among primary patients 5 died of disease in 1,4, 7, 8 and 12 months after diagnosis, one died in induction and two are alive on treatment (1 pt) or disease free (1 pt) with median OS of 7 months. **Conclusions.** Double-hit lymphomas are highly aggressive tumors with poor prognosis affecting, in our selection, predominantly woman and could be diagnosed in both primary and relapsed/progressive patients with acute lymphoblastic leukemia, follicular, mantle cell, diffuse large B-cell lymphomas and multiply myeloma. Diagnosis of these tumors is only possible on the basis of cytogenetic analysis. C-MYC translocations with IGH or IGL that are typical for Burkitt lymphoma are rare, and rearrangements with non-Ig chromosomal partners are predominated.

0695

ATYPICAL BCR-ABL1 REARRANGEMENTS DETECTED BY DNA SEQUENCING

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Background. BCR-ABL1 quimeric gene is detected in 90-95% of chronic myeloid leukemia cases (CML), in 20% of adult acute lymphoblastic leukemia (ALL), 5% of child ALL and 2% of acute myeloid leukemia (AML). During treatment, the quantification of BCR-ABL1 transcripts is important for disease monitoring. However, some cases show atypical rearrangements that are not detected by the primers usually used for the most frequent subtypes as: e13a2 (b2a2)/e14a2 (b3a2) that code 210kDa protein and e1a2, for the 190 kDa protein. **Aims.** The objective of this study is to relate the result of DNA sequencing to detect the variant cases. **Methods.** Cases in which Ph chromosome was detected by karyotype but no BCR-ABL1 rearrangement was found by real time PCR (RqPCR) were selected for the present study. Direct DNA sequencing was performed with primers for exons 1 (5'- CGG TTG TCG TGT CCG AGG -3') and 13 (5'- TTC AGA AGC TTC TCC CTG ACA T -3') in BCR gene and in exons 10 and 11 (5'- TCA TGG TCC AGA GGA TCG CTC -3') in ABL1 gene. After the RNA extraction from peripheral blood or marrow samples, reverse transcription and amplification by nested PCR of BCR-ABL1 transcript was performed, followed by direct sequencing using ABI PRISM 3130 (*Applied Biosystems*). **Results.** Five rare rearrangements were detected: e1a3 (58yr, male, AML), e13a3 (b2a3) (52yr, female, CML), e19a2 (46yr, female, ALL); while in a patient with CML (female, 29yr) another rare subtype, e13a4 (b2a4), was detected simultaneously to a frequent one, e13a2; and a deletion of exon 7of ABL (41yr, male, CML). **Summary and Conclusions.** BCR-ABL1 rearrangements in which BCR gene is juxtaposed to ABL1 exon 3 and not to exon 2 are rare. Apparently, only five cases of CML with subtype e1a3 and ten with e13a3 have been described so far. We add more five cases to these described. In summary, the association of different methods (RqPCR, karyotype and sequencing) was of fundamental importance to detect the rearrangement and plan the disease monitoring.

0696

IDENTIFICATION OF CHROMOSOMAL ABNORMALITIES BY MICROARRAY-BASED GENOMIC PROFILING AS COMPARED TO KARYOTYPING IN PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS)

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Background. Cytogenetic anomalies are routinely used as diagnostic, prognostic and therapeutic markers in the clinical management of myelodysplastic syndrome (MDS) (Greenberg *et al*. Blood 89:2079,1997). Although karyotyping is generally considered as the gold standard in the genetic diagnosis of MDS, 40-60% of the patients exhibit a normal karyotype. Intrinsically, the resolution of karyotyping is limited by its capacity to detect only those copy number changes that are microscopically visible (5-10 Mb in size). In contrast, microarray-based genomic profiling analyses allow a genome-wide detection of copy number alterations (CNAs), down to 100 kb in size, and regions of acquired uniparental disomy (UPD). Such analyses also overcome some of the other major limitations of karyotyping such as low success rate due to inadequate metaphase yield and/or poor banding quality. **Aims.** We have compared karyotyping and SNP-based microarray analyses with respect to the detection yield for genetic abnormalities in bone marrow samples of 87 MDS patients. **Methods.** For

microarray-based genomic profiling we used the 250k SNP array platform of Affymetrix. In order to rule out false positive results we used the following interpretation criteria: (i) the threshold for copy number aberrations was set at >5 Mb, (ii) smaller gains or losses were interpreted as aberrant in case they coincided with known (recurrent) aberrations as reported in the literature or in "Atlas of Genetics and Cytogenetics in Oncology and Haematology"(http://atlasgeneticsoncology.org/) and (iii) the threshold for aUPD was set at >10 Mb and to telomeric. Karyotyping and interphase FISH were performed using standard cytogenetic methods. **Results.** 47 of the 87 MDS patients had a normal karyotype. Of interest, in 7 of these 47 (15%) patients with a normal karyotype, acquired UPD and/or focal abnormalities (< 5 Mb) encompassing genes known to be involved in MDS tumorgenesis were observed. Through microarray-based genomic profiling, almost all numerical abnormalities as observed by karyotyping were detected. In addition, acquired UPD and focal (<5 Mb) CNAs were observed in the karyotypically abnormal group. Microarray-based genomic profiling also allows the identification of unbalanced translocations and a recently described phenomenon of genomic instability, fulfilling the definition of chromothripsis. As expected, balanced chromosomal abnormalities such as t(3;3)(q21;q26) and t(6;9)(p24;q34), which are present in a minority of MDS patients, were not identified. **Summary and Conclusions.** We demonstrate that microarray-based genomic profiling allows the identification of almost all copy number abnormalities also observed by karyotyping. In addition, we show that microarray-based genomic profiling allows the detection of novel focal CNAs and acquired UPDs in patients with both normal and abnormal karyotypes. The prognostic value of novel CNAs and acquired UPDs has to be evaluated in prospective clinical trials.

0697

GENOMIC BREAKPOINTS, LABORATORY AND CLINICAL FEATURES OF MLL-TET1 REARRANGEMENT IN ACUTE LEUKEMIAS: THREE NEW CASES AND LITERATURE REVIEW

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Background. Recently, rapid developments in new technology such as the whole genome/exome sequencing have revealed that some novel gene mutations such as *DNMT3A*, *IDH1*, *IDH2* and *TET2* contribute to the main process of leukemogenesis. Among these, *TET2*, one of the *TET* family gene located on 4q24, is becoming more important through its highly frequent mutation in various myeloid neoplasms such as myeloproliferative neoplasm (MPN), myelodysplastic syndromes (MDS), MDS/MPN and acute myeloid leukemia (AML). On the other hand, the *TET1* gene, located on 10q22, has been rarely reported as a partner gene for *MLL* rearrangement in AML and acute lymphoblastic leukemia (ALL) patients since its first report in 2002. **Aims.** There has been a total of nine documented instances of t(10;11)(q22;q23), where all except in two cases were detected in AML patients. Nonetheless, so far there has been little research with respect to the common features of the *MLL-TET1* rearrangement itself. Therefore the authors sought to provide a molecular, laboratory, and clinical characterization of the *MLL-TET1* rearrangement through literature review and three new AML patients. **Methods and Results.** Including three new cases, this study analyzes a total of 12 acute leukemia cases of t(10;11)(q22;q23) that are known so far, detected in 10 AML and 2 ALL patients. Six out of 10 AML patients (60%) have shown association with FAB-M4/M5 subtypes. There were seven male and five female patients and the median age was 38.0 (age range: 1 month ~ 67 years). Among nine patients with available chromosome study results, three patients have shown sole chromosomal abnormality of t(10;11), and the remaining six patients had accompanied various additional chromosomal abnormalities. *MLL-TET1* rearrangement was confirmed in eight out of 12 cases through molecular methods including Southern blot (1 case), long distance inverse-polymerase chain reaction (LDI-PCR) (6 cases), and reverse transcriptase-PCR (6 cases). The various genomic breakpoints of *TET1* gene in *MLL-TET1* rearrangement would be also described. **Conclusions.** As there were only six out of 12 patients with available clinical outcomes, this study may certainly lack sufficient amount of data. Nevertheless, the results show that four of them reached complete remission (CR). Meanwhile, three patients including one who did not reach CR died at an average of 18 months after initial diagnosis, showing little difference with the generally known poor prognosis of *MLL* rearrangements. Further clinical and molecular researches on acute leukemias with *MLL-TET1* rearrangement would be necessary. In the near future, the authors plan to conduct a functional study of the *MLL-TET1* fusion gene to know the role of the rare *MLL*-related fusion genes in leukemogenesis.

0698

THE WHOLE BLOOD MULTIPLEX DETECTION OF THE HERITABLE THROMBOPHILIA MUTATIONS F5-LEIDEN (F5G1691A) AND PROTHROMBIN (F2G20210A) USING A SIMULTANEOUS APPLICATION OF FLUOROMETRIC PRIMERS AND REAL-TIME PCR

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A full thrombophilia screen includes direct testing of *F*5G1691A and *F*2G20210A mutations, which increase a patient's risk to developing deep-vein thrombosis. The Molecular Hematology Laboratory, at the John Radcliffe Hospital, analyzes 2,000 thrombophilia samples per-year. These mutations are routinely detected via a multi-stage process of: DNA extraction, PCR amplification, over-night restriction and interpretation by gel-electrophoresis. This method is robust, reliable and cost-effective for weekly, batched assays. However, the nature of batched assays causes a two-day method to generate turnaround times of up to two weeks. DNA extraction and long-term storage is costly.The 3M™ Integrated Cycler was evaluated to determine if this new technology could provide service improvement. The simultaneous application of real-time PCR and fluorescently labelled primers, produces a method which offers the possibility of detecting these mutations from whole-blood, within two hours. This rapid processing time is due, in-part, to the removal of a lengthy DNA extraction process.The 3M™ Integrated Cycler utilizes the Focus Simplexa™ direct chemistry to monitor PCR amplified fluorescence levels of four independent primer pairs. These primers are specific to *F*5 and *F*2 wild-type and mutant genotypes. The intensity of the fluorescence is quantified by four dedicated lazer-channels, over the course of 45 PCR cycles and is then compared to established thresholds; C_T values are based on a panel of control samples.This Simplexa™ kit contains four sets of primer pairs and a master-mix, which must be combined specifically for each run. This system also requires a one-part patient sample and three-parts PBS buffer dilution to be made before use. The primer-mix and samples are pipetted into the universal-disk, as indicated by the program software. Once the disk is loaded, it is sealed and placed onto the 3M™ Cycler for analysis using pre-programmed cycling conditions.Thirty-three samples of known genotypes were used to establish the initial whole blood C_T values. To audit the strength of these reference thresholds against the gel-electrophoresis method, a whole-blood, blind panel of 46 patient samples, 6 known positive controls and 2 negative controls were used; totalling 78 individual results for each SNP, over four separate runs.Two separate samples were run five times in the same assay and a further six samples were run four times in different assays, to determine the intra and inter-assay variation of C_T. The C_T values showed maximum results of 4.0% and 4.4% respectively; both in the *F*2 wild-type detection channel. There is a positive correlation of 97% between the 3M™ Cycler and the gel-electrophoresis method.Once the threshold limits were established, the 3M® Cycler was capable of identifying the *F*5G1691A and *F*2G20210A in wild-type, heterozygous and homozygous states. In this setting, where DNA samples are not needed for further investigation, family studies or archive, the assay may be useful for the rapid, simultaneous detection of the two SNP's. Further examination is required to establish sample acceptance criteria, including: white-cell-count, sample-condition and anticoagulant-status, and their corresponding effect on C_T levels.

0699

FANCONI ANEMIA FOUNDER MUTATION IN MACEDONIAN PATIENTS

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Fanconi anemia (FA) is a rare autosomal recessive disorder (1-5/1 000 000 live births) genetically and phenotypically heterogeneous, defined by cellular hypersensitivity to DNA cross-linking agents. Clinically FA is characterized by variable developmental abnormalities that may affect skeletal morphogenesis as well as any of the major organ systems, stem cell loss, causing progressive bone marrow failure (BMF) and sterility, and profound predisposition to neoplasia mostly leukemia and solid tumors. FA is the most frequent inherited cause of BMF.The FA complementation system consists of 15 FANC genes. There are evidences that all 15 gene products operate in a common molecular pathway

to preserve genomic integrity. Biallelic disruption of any one of these genes results in this clinically defined syndrome. The most prevalent ones are FANCA, FANCC, FANCG, and FANCD2. At the molecular level, a fundamental defect in DNA repair underlies this complex phenotype. FA-A is the most common group, accounting for approximately 65% of all affected individuals. The mutation spectrum of the FANCA gene, located on chromosome 16q24.3, is highly heterogeneous, including point mutations, small insertions/deletions, splicing mutations, and large intragenic deletions. FA-A is usually associated with private *FANCA* mutations in individual families. Thus, the number of different pathogenic variants described for the *FANCA* gene is very high considering the relatively low number of patients. Here we describe three patients (2 females, 11 and 17 years old and 1 male, 23 years old) with Fanconi anemia with similar clinical presentation: BMF, ren arquatus and renal ectopy and abnormal skin pigmentation *(café-au-lait)* without skeletal abnormality. In one of the female patients the persistent Ductus arterious, vesico-ureteral reflux and ptosis were also present. The molecular analysis of FANCA gene using the MLPA kit Fanconi anemia P-031, showed homozygous deletion of exon 3 in all three patients. Molecular analysis of the flanking regions of exon 3 precisely defines unique deletion of 2040 bp and insertion of C (1788_3824insC). The breakpoints are in intron 3 and 4 in the Alu repetitive sequence. We conducted a familial analysis and proved that the mutation was inherited from the parents. These are the first three patients homozygous for deletion of ex3 described to date. The patients are not related but they came from the same region from Macedonia. Determination of family pathogenic variants is the ultimate confirmation of diagnosis and is necessary for molecular prenatal or preimplantation tests and mutation carrier detection. The homozygous deletion of exon 3 of *FANCA* gene is a founder mutation of Fanconi anemia patients inMacedonia. Our finding has very strong implication in diagnostic and carrier screening strategy for BMF and Fanconi anemia in Macedonian patients and in comprehensive genetic counseling.

0700

IDENTIFICATION OF THE FIRST MUTATION IN BRE MOTIF OF β-GLOBIN GENE AND ITS POLYGENIC INHERITANCE WITH TWO OTHER β-GLOBIN MUTATIONS IN PATIENTS OF THE SAME FAMILY

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Background. Thalassemia is one of the most common inherited genetic diseases in the Mediterranean region. Patients with heterozygous mutations in beta thalassemia, and to a lesser extent alpha-thalassemia, suffer from mild microcytic anemia. While the anemia is mild and confers no clinical threat in carriers, homozygosity of the mutation often leads to major thalassemic disease. Mutations and variants of α-globin and β-globin chains usually segregate independently, and there are rare reports of co-inheritance. Clinical outcomes of β and α-globin homozygous mutations vary significantly. **Aims.** A 7 year-old boy presented to our clinic with persistent mild microcytic anemia and recurrent infections, but with repeatedly normal iron profile and with normal HPLC . This prompted further genetic investigation of underlying β-and α-globin gene mutations. **Methods.** Upon receiving IRB approval and parents' consent, DNA samples were extracted from blood withdrawn from proband, parents and sibling. Genetic analysis with multiplex gap-PCR tests were conducted in the Chronic Care Center, Lebanon and reconfirmed in Boston University, USA for single a-globin gene deletions of rightward ($-a^{3.7}$) and leftward ($-a^{4.2}$) types, deletion of two a-globin genes in cis of the($—^{MED}$) and $-(\alpha)^{20.5}$, as well as for β-globin gene single point mutations and single nucleotide polymorphisms . **Results.** The father's a-globin genotype was (aa^{Hph}/ aa), while the mother's a-globin genotype showed another mutation which was ($-a^{3.7}$/aa). The a^{Hph} mutation is a deletion in the IVSI donor splice site which is a 5-base pair deletion of (-GTGAG) in a2-globin genes. The $a^{3.7}$ deletion is a single a-globin deletion and is the most common form of a-thalassemia mutation in the US and the Asia. The proband and his sibling inherited both a-globin mutations from each parent, concomitantly, and their a-globin genotypes were ($-a^{3.7}$ / a^{Hph}a). While the father's β-globin genotype was normal, the mother had a novel mutation (G>C) at nucleotide (-39) in BRE motif. Notably, genetic analysis showed that the proband and his sibling also inherited the β-globinmutation (G>C) from the mother. A list of mutations in all four members of the family is found in Table 1. **Conclusions.** This is the first report to show a mutation in BRE motif, where TFIIB binds to the β-globin gene promoter. Moreover, the ($-a^{3.7}$/ aa) genotype segregated with the BRE-motif mutation of β-globin in the mother and both siblings. The polygenic segregation, although rare in beta and alpha thalassemias, led to a mild microcytic anemia without iron deficiency and normal HPLC. Moreover, carrying two different a-globin mutations in proband and sibling conferred no fulminant a-thalassmia disease, despite a concomitant β-globin mutation. Further studies to decipher the clinical significance of this novel mutation in BRE motif of β-globin gene are warranted.

Table 1. Distribution of mutations in a- and -globin genes among family members.

	Father	Mother	Brother	Proband
β-Globin genotype	Normal	Hetero Nt –39 G>C mutation	Hetero Nt –39 G>C mutation	Hetero Nt –39 G>C mutation
α-Globin genotype	$\alpha\alpha$ / $\alpha^{Hph}\alpha$	$-\alpha^{3.7}$ / $\alpha\alpha$	$-\alpha^{3.7}$ / $\alpha^{Hph}\alpha$	$-\alpha^{3.7}$ / $\alpha^{Hph}\alpha$

0701

A CERTIFIED PLASMID REFERENCE MATERIAL FOR THE STANDARDISATION OF BCR-ABL1 MRNA QUANTIFICATION BY REAL TIME QUANTITATIVE PCR

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Background. Serial quantification of *BCR-ABL1* mRNA is an important therapeutic indicator for patients with CML and Philadelphia-positive ALL, but there is substantial variation in the qRT-PCR protocols employed by testing laboratories. The principal difference is the use of different internal control genes (CG) to normalise results . For positive specimens, raw results are expressed as a ratio of *BCR-ABL1/CG* transcripts whereas for negative specimens the number of CG transcripts indicates the sensitivity with which *BCR-ABL1* can be excluded for that sample. Determination of the number of *BCR-ABL1* and *CG* transcripts is typically performed by reference to a plasmid calibrator, however different calibrators are in use worldwide and currently no standard exists to which they can be aligned. Comparative studies have demonstrated that the use of different plasmid calibrators and different batches of the same calibrator give rise to significant variation in assay results. **Aims.** To help standardise the reporting of copy numbers of *BCR-ABL1 (e14a2)* and CG (*ABL1*, *BCR* and *GUSB*) transcripts we sought to develop an internationally accepted plasmid certified reference material (CRM). **Methods.** Fragments specific for the fusion transcript *BCR-ABL1 e14a2* and control genes *BCR* and *GUSB* were amplified and cloned into pUC18 in a 1:1:1 ratio. For the CRM production according to ISO Guide 34:2009 by the IRMM, the plasmid was linearised, diluted to six different concentrations and copy numbers were assigned following 18 independent digital (Fluidigm) PCR measurements results for each dilution. For confirmation of the identity the entire plasmid was sequenced. The material was characterised for inter unit-heterogeneity, stability during dispatch and storage in accordance with ISO Guide 35:2006. A commutability study to assess the utility of the six CRMs was performed by nine *BCR-ABL1* testing laboratories using the six plasmid dilutions, two common cDNAs and their own local methods. **Results.** The copy numbers of the 6 calibrants were assigned as $1.08 \pm 0.12\times10^6$, $1.08 \pm 0.11\times10^5$, $1.03 \pm 0.10\times10^4$, $1.02 \pm 0.09\times10^3$, $1.04 \pm 0.10\times10^2$ and 10.0 ± 1.5 copies/µl (with a coverage factor $k = 2$ used for the expanded uncertainty . Data from the commutability study assessing the utility of the CRM were collated and the calibration standard curves were evaluated for their gradient and coefficient of determination ($r2$). In total, 171/174 (98.3%) calibration curves showed acceptable gradients (range -3.0 to -3.6); *BCR-ABL1* (n= 84), *ABL1* (n=*36*), *BCR* (n=*23*) and *GUSB* (n=*28*). 3/174 (1.7%) calibration curves were rejected because their gradient was less than -3.60 (-3.61 (n=1), -3.62 (n=1). All calibration curves had an $r2$ above 0.99. The CRMs proved to be commutable for all methods employed. **Summary.** We conclude that this set of 6 plasmid CRMs with defined DNA copy numbers can be used to standardise the reporting of transcript numbers of *BCR-ABL1* and three control genes; *ABL1, BCR* and *GUSB* in *e14a2* RQ-PCR monitoring tests. The plasmid dilutions with the CRM code ERM-AD623a-f, can be obtained from the IRMM or its authorised distributors (http://irmm.jrc.ec.europa.eu).

0702

COMPARISON OF THE BCR-ABL GENE EXPRESSION BY THE GENEXPERT SYSTEM AND STANDARDIZED RQ-PCR METHOD IN THREE POLISH MOLECULAR LABORATORIES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TKIS

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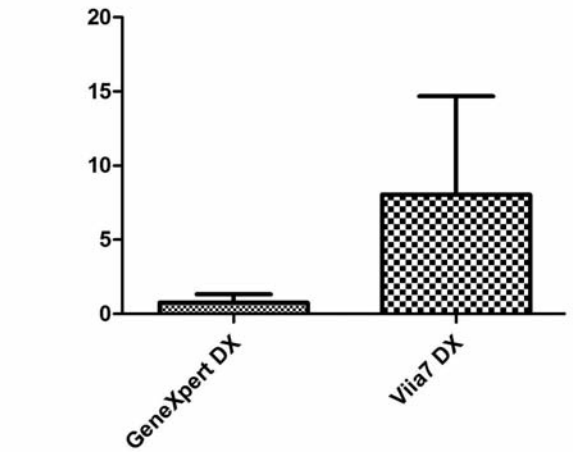
Background. The GeneXpert System was introduced to facilitate the molecular monitoring of therapy with tyrosine kinase inhibitors (TKI) of patients suffering from chronic myeloid leukemia. **Aims.** The aim of the study was to evaluate the novel rapid PCR GeneXpert System with internationally standardized *BCR-ABL1/ABL1* RQ-PCR methodology **Methods.** The comparison of the *BCR-ABL1* gene expression consisted of paired *BCR-ABL1/ABL1* expression analysis performed in three Polish Molecular Laboratories by RQ-PCR TaqMan methodologies aligned to International Standard (IS) and by the GeneXpert *BCR-ABL* System. 90 patients with chronic phase chronic myeloid leukemia expressing e13a2 or e14a2 *BCR-ABL1* transcripts and treated with tyrosine kinase inhibitors were evaluated. Patients were subdivided into three groups according to BCR-ABL1 transcript level estimated by standardized RQ-PCR method. Group 1 consisted of patients with *BCR-ABL1/ABL1* ratio below 0,1% [IS], group 2 with *BCR-ABL1/ABL1* ratio between 0,1% and 1,0% [IS], and group 3 with *BCR-ABL1/ABL1* ratio between 1,0% and 10,0% [IS]. **Results.** The comparable results of two evaluable techniques were observed in 32% of all samples. The Wilcoxon test revealed statistically significant differences between the results of GeneXpert System and standardized RQ-PCR *BCR-ABL1/ABL1* method (p< 0,0001) (Figure 1). The mean *BCR-ABL1/ABL1* gene expression evaluated by the GeneXpert System was 1 log lower than in the RQ-PCR method. The comparable results of both techniques in group 1, 2 and 3 were noted in 35%, 15% and 17% respectively. The differences were statistically significant in each evaluated group. **Conclusions.** The GeneXpert system provides very easy to use and rapid diagnostic platform, which could be very useful in evaluating CML patients' response to TKI therapy. Improvement of system sensitivity and the development of proposed already a reagent lot-specific conversion factor to the international standard could further enhance the reliability of results obtained by the use of GeneXpert in patients with CML monitored for the response to TKI therapy.



Wilcoxon signed rank test	
P value	P<0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of positive, negative ranks	3.000 , -858.0

Figure 1.

Hematopoietic stem cells and microenvironment 2

0703

THE PHENOTYPE OF EARLY ERYTHROID CELLS PARALLELS WITH THEIR GENE EXPRESSION PROFILE

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Background. Our current knowledge about the regulation of erythropoiesis is based on cell culture techniques using various cytokines, immunological methods such as flow cytometry, recent molecular techniques like gene expression profiling and animal models. However, the different stages of the early erythropoietic cells cannot be clearly delineated so far by flow cytometry nor by molecular analyses. **Aims.** The aim of our recent study was to investigate the gene expression profile of distinct stages of the erythroid differentiation separated by high-sensitive flow cytometry. **Material and Methods.** Bone marrow MNC from twenty patients without marrow involvement of an underlying disease and from six healthy volunteers were obtained after signing an informed consent. A backbone consisting of CD34, CD117, CD45, CD105 and CD71 was established for a ten colour FCM (Aria III cell sorter, Becton Dickinson) and used in combination with additional markers (CD13, CD33, HLA-DR, CD235a, CD238, CD239, CD36, CD19, CD14, CD 11b, CD38, CD173) to distinguish the different early erythroid precursor cells. FACSDiva software Version 6.1.3 (BD Biosciences) was used for the evaluation of the multicolour analysis. Pure (> 95%) subsets were obtained by flow cytometric sorting with specific marker combinations.RNA from 40.000 to 1.000.000 cells of each subset were extracted using the miRNAeasy kit (Qiagen) for the gene expression analysis, afterwards cDNA and double strand biotin labeled cRNA production samples were purified, fragmented and hybridized to the GeneChip U-133 Array (Affymetrix). After scanning, the normalization of raw data was performed in Partek software. **Results.** Using the ten colour FCM, four different stages of the early erythropoiesis could be delineated. The earliest compartment is the common progenitor stage (CD34+, CD117+, HLA-DR+, CD105-, CD71-, CD36-). The stage 2 can be defined by the occurrence of the CD71 (transferrin receptor) expression. At this stage of differentiation CD 34 and CD117 remain detectable, whereas all myeloid associated structures are no more present. Within stage 3 erythroid-specific antigens appear (CD105, CD173, CD238) while some antigens of the progenitor stage decrease (CD34 and HLA-DR). In stage 4 most blood group antigens are expressed whereas CD117 is lost.Gene expression analysis revealed a strong correlation for the expression level of most relevant markers and blood group antigens. A significant (p-value <0,01; fold change >2,0) up-regulation was found for 519 genes in CD105 positive erythroid precursor cells (stage 3 and 4) in comparison to the common progenitor level, whereas the expression level of 1520 genes was significantly reduced. Furthermore, significant differences between the erythroid stages 1 to 4 were detectable in all pathways studied (erythropoietin-receptor, BMP/Wnt, TGF, TNF) including almost all transcription factors responsible for the erythroid differentiation and functional relevant genes for hem-synthesis and iron metabolism. **Conclusions.** Using highly sensitive FCM different compartments of the erythroid development can be delineated, which enables further characterization of these populations by gene expression analysis. The results of this approach represent the basis for a systematic comparison with pathological conditions involving the early erythroid differentiation.

0704

CYTOMEGALOVIRUS INFECTION IMPAIRS IMMUNOSUPPRESSIVE AND ANTIMICROBIAL EFFECTOR FUNCTIONS OF HUMAN MULTIPOTENT MESENCHYMAL STROMAL CELLS - IMPLICATIONS FOR THEIR CLINICAL USE

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Background. In addition to their multilineage differentiation potential human multipotent mesenchymal stromal cells (MSC) provide pleiotropic immunosuppressive functions that are partly mediated by expression of the tryptophan-

catabolizing enzyme indoleamine-2,3-dioxygenase (IDO). Moreover, upon stimulation with inflammatory cytokines MSC exhibit broad-spectrum antimicrobial effector function directed against various clinically relevant pathogens and these effects are also IDO-dependent. This dual immunosuppressive and antimicrobial properties render MSC a promising novel cellular immunosuppressant which is currently under intensive clinical investigation in various auto- and alloimmune diseases such as steroid-refractory graft-versus-host-disease (GvHD) after allogeneic hematopoietic stem cell transplantation, Crohn's disease and multiple sclerosis. However, recent data indicate that signals from the microenvironment including those derived from microbes may modulate MSC effector functions e.g. via activation of toll-like receptors. **Aims.** As human cytomegalovirus (HCMV) represents a prominent pathogen in immunocompromised hosts, in particular in patients with GvHD, we here investigated the impact of HCMV infection on immunosuppressive and antimicrobial activity of human MSCs. **Methods and Results.** Human bone marrow-derived MSCs were infected with HCMV and their T lymphocyte inhibitory capacity was assessed in co-culture with anti-CD3-stimulated human peripheral blood lymphocytes. While uninfected MSCs substantially impaired T cell proliferation, HCMV infection significantly reversed this T cell inhibitory effect of human MSCs ($p<0.05$). In additional experiments we found that the inhibitory effect of MSC on T cell proliferation required a minimum amount of MSC present in the co-culture system. While the addition of HCMV to cultures with high numbers of MSCs antagonizes their T cell inhibitory effect, the same amount of HCMV added to low number of MSCs did not alter T cell proliferation, thus ruling out an unspecific T cell stimulatory effect of HCMV. In subsequent analyses, we observed a substantial negative impact of HCMV infection on cytokine-induced antimicrobial effects of MSCs which are directed against bacteria such as *Staphylococcus aureus* and intracellular parasites such as *Toxoplasma gondii*. We then went on to analyse the potential impact of HCMV infection on IFNγ-induced IDO expression as one of the key mechanism of MSC-mediated T cell suppression and antimicrobial effects. In these experiment we found a substantial impairment of IDO expression after HCMV infection of MSCs in western blot analysis as well as enzyme activity assays. Moreover, this interaction between HCMV and IDO-mediated effects critically depended on intact virus, the time point of CMV infection as well as the numbers of MSCs and virus employed. **Summary and Conclusions.** Our results provide first evidence that HCMV infection critically impairs immunosuppressive and antimicrobial effector functions of human MSCs via interaction with the IFNγ-induced IDO pathway. As a consequence, overt HCMV infection of MSC recipients might undermine the clinical efficacy of MSC treatment. Thus, antiviral treatment should seriously be considered in patients with active HCMV infection prior to MSC administration.

0705

ALTERATIONS IN BONE MARROW STROMAL PRECURSOR CELLS IN PATIENTS BEFORE AND AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Stromal damage and recovery in allogeneic hematopoietic stem cells transplant (alloHSCT) recipients is obscure. **Aims.** The aim of the study was to investigate the dynamics of stromal precursor cells - multipotent mesenchymal stromal cells (MSC) and colony forming unit fibroblasts (CFU-F) - in the bone marrow of patients during one year after alloHSCT. **Methods**. AlloHSCT was performed to 12 patients (6 male and 6 female): 3 patients with CML, 5 with AML, 3 with ALL and 1 with B-CLL. Conditioning was myeloblative in 8 patients and reduced intensity in 4. After informed consent bone marrow was aspirated: before conditioning, and 30, 60, 90, 120, 180-240 and 360 days after transplantation at the moment of control clinical observations. MSC were cultured in alfaMEM media supplemented with 10% fetal calf serum. Cumulative MSC production was counted after 5 passages. CFU-F concentration in bone marrow samples was analyzed in 14 days.*Results* The growth parameters of patients' and donors' MSC are significantly different. Even before alloHSCT average time to passage is 1.5 fold longer in patients' MSC (9.0 ± 0.8 days) than in donors' ones (6.6 ± 0.4 days). In 30 days after alloHSCT average time to passage increased 3 fold in comparison with donors' (20.6 ± 5.0 days), than it gradually decreased remaining longer than before alloHSCT up to one year. Before transplantation cumulative cell production of MSC is more than ten times lower than in donors' MSC. In 30 days after alloHSCT cumulative cell production decreased 1000 fold, than increased slowly, but at the day 120 it was 10 fold lower than at the day 90. Only in one year it attends basic level.Concentration of CFU-F in the bone marrow of patients before transplantation was 1.3 fold lower than in the bone marrow of donors. In 30 days after alloHSCT concentration of CFU-F in bone marrow of patients decreased more than 4 fold (29.6 ± 7.9 per 10^6 bone marrow cells before alloHSCT and 7.2 ± 3.1 per 10^6 - 30 days after). Further it gradually increased and one year after alloHSCT mount to 75% (22.4 ± 14.8) of value before transplantation. No differences were revealed between patients after myeloablative or reduced intensity conditioning regimen. **Conclusions**. The results demonstrate that stromal precursor cells - MSC and CFU-F are altered in patients after alloHSCT. MSC from patients have reduced proliferative potential in comparison with MSC from donors which does not restored even in one year after alloHSCT. So the data suggest that stromal cells are violently damaged by alloHSCT procedure and do not regenerate completely even in one year. The status of hematopoietic microenvironment could influence the hematopoiesis in patients after alloHSCT. Further investigations of stromal precursor cells are needed.

0706

CDK6 IN HSC HOMEOSTASIS

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Background. Cell cycle deregulation is a common feature of cancer (Cordon-Cardo 1995; Malumbres and Barbacid 2001; Deshpande, Sicinski et al. 2005; Kim and Diehl 2009). The G1-kinase, CDK6, is frequently expressed at high levels in human lymphoma and lymphoid leukemia (Chilosi, Doglioni et al. 1998; Lien, Lin et al. 2000; Schwartz, Engel et al. 2006; Nagel, Leich et al. 2008). Additional roles - apart from promoting cell cycle progression - have been postulated for CDK6; it has been for instance posited that CDK6 regulates differentiation in several cell types (Grossel and Hinds 2006). CDK6 deregulation in mice results in an altered composition of lymphoid cell subsets as well as hematopoietic stem and progenitor cells (Hu, Deshpande et al. 2009; Kollmann et al. 2011; Hu et al. 2011). **Aims.** With this study we want to analyze the impact of CDK6 on lineage differentiation as well as haematopoietic stem cell (HSC) homeostasis and if the efficacy of bone marrow transplantation depends on CDK6 kinase activity. A disturbed HSC homeostasis may play an important role in leukemia/lymphoma formation. Accordingly, CDK6 represents a potential target for the development of therapeutic drugs. **Methods and Results.** When we analyzed cell lineages in haematopoietic organs (bone marrow, spleen and blood) by FACS, we found one supplemental consistent alteration in *Cdk6-/-* mice. In mice lacking CDK6 the granulocytic population was significantly reduced in bone marrow and blood compared to control mice. To test if the lack of CDK6 influences HSC populations we analysed the HSC-fractions in 4- to 5-week-old *Cdk6-/-* mice by FACS. We found a significant increase of lineage negative cells as well as a higher percentage of HSCs, detectable in the bone marrow of *Cdk6-/-* mice. In contrast the percentage of myeloid progenitors (MPs) was decreased. To determine the reconstitution ability of *Cdk6-/-* bone marrow, we transplanted isolated *Cdk6-/-* and *Cdk6+/+* bone marrow cells into lethally irradiated mice. Successful engraftment was detected in both groups, albeit significantly less Lin^- cells were found in mice reconstituted with *Cdk6-/-* BM compared to the *Cdk6+/+* mice. **Summary.** These results indicate that CDK6 is involved in hematopoiesis at multiple levels including alterations of HSCs.

0707

PPARG INHIBITOR PROMOTES HEMATOPOIETIC RECOVERY AFTER CHEMOTHERAPY

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Background. Increased fat fraction within bone marrow microenvironment induced by high-dose HD chemotherapy used to be observed though its role in hematopoietic recovery was unclear. PPARγ has been considered as a vital transcriptin regulator of adipogenensis *in vitro* and *in vivo* in models of diabetes. BADGE, as an inhibitor of PPARγ, has been showed to successfully prevent bone marrow adipocytes formation after lethally irradiated, resulting a faster hematopoietic engraftment. Whether adipocytes play a negative role in hematopoietic recovery after high-dose chemotherapy remains unknown. **Aims.** We wonder if PPARγ inhibitor can prevent the adipogenesis induced by HD chemotherapy and then improves hematopoietic recovery. **Methods.** 6~8 week-old C57BL/J mice were treated with Arac in order to induce bone-marrow adipocytes formation. BADGE was administrated daily since the day Arac treated while DMSO was used as the control. Mice were bled every week for peripheral blood analysis and femurs and tibias as well as tail vertebraes were detached for pathological HE sections. BM cells were flushed out for further

experiments including RT-PCR ,colony forming unit (CFUs), flow cytometer (FACs) and magnetic active cell sorting (MACS). **Results.** Fat hyperplasia appeared in the control group since one week after HD chemotherapy which can be restrained in the BADGE-treated group. After 2 weeks BADGE treatment, peripheral white blood cells increase 30%~40% over the control group while the neutrophils were 1.5~2 times higher. CFUs of BM cells increased by 25%~30%. Meanwhile, compared with the control group, though HSCs (ckit+Lin-Sca1+) seemed no obvious difference between the two groups, percentage of hematopoietic progenitor cells (HPCs, ckit+Lin-Sca1-)sorted by MACs were 2 times higher. Further more, the proportion of Ki67 (+) CD45 (+)BM cells and Ki67 (+) LSK HSCs analyzed by FACs were significantly increased by 1.5~2 times. **Conclusions.** Fat hyperplasia within BM induced by HD chemotherapy can be restrained by PPAR γ inhibitors, resulting an improvement of hematopoietic recovery after the hematopoietic stress.

0708

PHYSIOLOGICAL AND PROTEOMIC ASPECTS OF FUNCTIONAL CORD BLOOD CD34+ CELLS MAINTENANCE AT 4°C UNDER HYPOXIA AND HYPERCAPNIA

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Background. The short-term conservation of hematopoietic stem and progenitors cells is actually performed in hypothermia (+4°C). In nature, long-term survival of some animals in hypothermia is enabled by regulated metabolic depression which can be induced by their exposure to low O_2 (hypoxia) and increased CO_2 (hypercapnia) environment. Similarly, in physiological conditions, the primitive hematopoietic cells are maintained in a hypometabolic state in the poorly oxygenated bone marrow niche. Recently, we demonstrated that the same protective mechanism is operational in mobilized peripheral blood primitive hematopoietic progenitors and stem cells during their storage at4°C (Jeanne et al, Transfusion 2009). **Aims.** The aim of this study was to test if exposure to hypoxic/hypercapnic gas mixture could be also beneficial for the preservation of functional cord blood $CD34^+$ hematopoetic progenitors in hypothermia and, if so, to analyse physiological and proteomic aspects of this phenomenon. **Methods.** Cord blood $CD34^+$ cells were incubated in conservation medium Stem-α S3 for 10 days at +4°C under hypoxic (1 and 5% O2) and hypercapnic (2,5 and 9% CO2) concentration or air (20% O_2 and 0,05% CO_2). The functional maintenance of committed hematopoietic progenitors (CFC assay) and stem cells (Scid-Repopulating Cells (SRCs) assay estimated by the presence of human markers [CD45, CD19, CD33] and human CFC in NOG/Scid mice femur 6 weeks after transplantation) in parallel with a flow-cytometry analysis of phenotype and vital functions (Aldefluor, AnnexinV/PI, ATP bioluminescent assay, DiBAC4, TMRM, H2DCFDA staining). Proteomic analysis was performed using iTRAQ labelling followed by peptide fractionation (SXC chromatography) and mass spectrometry (LC/MS/MS). **Results.** Incubation in hypoxia and hypercapnia doubled the survival $CD34^+$ cells (60%) comparing to air (30%). Similar ratio is obtained for CFC (34±9% vs 18±6% in air). $5\%O_2/9\%CO_2$ ratio appeared to be the optimal hypoxic/hypercapnic combination. Better cell maintenance in this condition was associated with a higher frequency of primitive a*ldehyde dehydrogenase* (ALDH) expressing cells and SRCs. These cell-protective effects seem to concern a better preservation of the plasma and mitochondrial membrane potential. Hypoxia/hypercapnia also ensures maintenance of viable cells and induces less apoptosis, in spite of a production of deleterious ROS and a drop of ATP amount/per viable cell which were equivalent to that obtained in air. These effects seem to be principally mediated via hypercapnia. Proteomic study revealed that overall protein's content was better preserved in hypoxia/hypercapnia. In addition, this analysis enabled to identify and to distinguish proteins sensitive and insensitive to hypothermia irrespective to the gas phase, as well as the proteins contributing specifically to the hypoxia/hypercapnia cell protective effect. Among them are some protein families known to be implicated in the long time survival of hibernating animals in hypothermia. **Conclusions.** Present work demonstrates the critical physiological effects and indicates protein candidates implicated in hypoxia/hypercapnia-mediated functional maintenance of hematopoietic progenitors in hypothermia. These results suggest a way to optimise cell conservation without freezing and to design a new generation of conservation media.

0709

CYTOKINE PROFILE AND EFFECT OF ALLOGENIC BONE MARROW MESENCHYMAL STEM CELLS ON PROLIFERATION OF NORMAL HEMATOPOIETIC PROGENITORS AND LEUKEMIC CELLS

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Background. Currently, immunological properties of MSCs have found a greater therapeutic use in transplantation to improve engraftment, treatment of GVHD and in regenerative medicine. MSCs have the capacity to maintain hematopoiesis (through the production of G-CSF, GM-CSF), immunomodulation properties (through the production of IFN-γ, IL-2, IL-6, IL-1β, TNF-α and chemokines - IL-8, MCP-1, MIP-1β) and stimulation of reparative processes (through the production of VEGF, FGF-basic, IGF-1, IL-6, TIMP-1/MMP-9). MSC has impact to leukemic cells present in bone marrow of the recipient in the form of minimal residual disease. **Aims.** to investigate the mechanism of the effect of allogeneic bone marrow MSCs on the ability of tumor cells to spontaneous and induced apoptosis. We also assessed the cytokine profile of MSCs according to the time of cultivation. **Methods.** Leukemic cells from bone marrow samples of patients (age 6 months - 17 years) with newly diagnosed acute leukemia (AL): B-ALL-45, T-ALL-13, AML-19. MSC (from 54 healthy donors) were cultured in DMEM with 20% FCS. Clonogenic study of granulocyte-macrophage bone marrow progenitors (from 15healthy donors) were performed in semisolid agar medium. The sensitivity of blast cells to cytotoxic drugs was studied by the MTT-assay. To determine the levels of spontaneous and induced apoptosis in leukemia cells we use Apoptosis Detection Kit II (BD). Cytokines produced by MSCs from the bone marrow was determined by flow Cytofluorometer using reagents BD Cytometric Bead Array early and late passages. **Results.** MSCs stimulate colony formation of granulocyte-macrophage precursors, surpassing the effectiveness of phytohemagglutinin-leukocyte conditioned medium as a source of colony stimulating factors. Efficiency of cloning was 27.9 (SD 1.5) and 22.9 (SD 2.4), respectively. When leukemic cells were cultured on MSC for 4 days the proportion of viable cells was higher at 47.2% in ALL, and 63.1% in AML compared with controls. Incubation of leukemic cells with MSCs resulted in a decrease of sensitivity to cytarabine in 2 times in B-ALL, in 1.5 times in AML and 6 times in T-ALL. Under the influence of MSCs sensitivity of leukemic cells to daunorubicin decreased in all groups. MSC increased the sensitivity of blast cells from ALL patients to MP, but did not influence the sensitivity of AML blast cells to MP. MSCs inhibit apoptosis of leukemic cells induced by cytarabine. The study of MSCs cultures with CBA showed that MSCs produce a wide range of cytokines (IL-2, 4, 6, 10; FGF, VEGF), which may mediate the effect of MSCs on the viability of hematopoietic progenitors and leukemic cells. Some changes in production of various cytokins depending on time of cultivation have been observed. **Conclusions.** On the basis of the research is clear that the important aspect is the safety of MSCs in terms of their influence on the sensitivity of leukemic cells to chemotherapy in patients with hematologic malignancies. The functional capacity of MSCs, which is realized through the production of a wide variety of cytokines, may play an important role in the regulation of immunological, reparative and other processes.

0710

THE NUCLEAR FACTOR NF-YA IN HUMAN BONE MARROW MESENCHYMAL STEM CELLS: AN EMERGING ROLE IN ADIPOGENESIS

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Background. The nuclear transcription factor Y (NF-Y) is a heterotrimeric CCAAT-binding transcription factor consisting of the subunits A, B, and C that appears to have a complex role in hematopoietic stem cell (HSC) self-renewal and differentiation along different hematopoietic cell lineages. No available data, however, exist on the role of NF-Y in cells of the non-hematopoietic bone marrow (BM) stroma, namely the mesenchymal stem cells (MSCs) and their progeny. **Aims.** To evaluate mRNA expression and protein levels of the main regulatory NF-Y subunit, namely the NF-YA, throughout long-term BM-MSC cultures and probe its possible involvement in BM-MSC differentiation process towards the adipogenic and osteogenic lineages *in vitro*. **Methods.** MSCs were isolated from BM aspirates of hematologically healthy subjects undergoing orthopedic surgeries after informed consent. MSCs were *in vitro* expanded until passage (P)-10. P3 BM-MSCs were *in vitro* induced to adipogenic or osteogenic differentiation according to standard protocols. Differentiation were

verified by the appropriate cytochemical stains (Oil-Red O and Von-Kossa, respectively) and by evaluating the relative gene expression of lineage-associated markers by real-time PCR, namely PPARG, CEBPA, LPL for adipogenesis and RUNX2, DLX5, ALP, OSX, OSC, BSP for osteogenesis. All PCR results were expressed as $2^{-\Delta Ct}$. To knock-down NF-YA expression, P3 BM-MSCs were transduced with NF-YA shRNA or control (irrelevant shRNA) lentiviral particles. Protein levels of NF-YA were immunodetected by Western blots. **Results.** A statistically significant reduction in mRNA levels of NF-YA was found in P10, as compared to P3 BM-MSC cultures (n=9) (0.002±0.0007, and 0.004±0.001, respectively, *P*=0.0296). During adipogenic differentiation (n=12) a significant increase in NF-YA transcription levels was observed at day6 (0.007±0.003, *P*=0.0452) and day12 (0.01±0.005, *P*=0.347) as compared to the onset of differentiation induction (0.004±0.002). Moreover NF-YA expression levels strongly correlated to CEBPA (*r*=0.751, *P*=0.001), PPARG (*r*=0.705, *P*=0.003), and LPL (*r*=0.712, *P*=0.003) mRNA levels. In accordance to mRNA levels, protein levels of NF-YA also increased during adipogenesis. In contrast, during osteogenic differentiation (n=6) no statistically significant change was detected in NF-YA mRNA expression or protein levels. To evaluate whether NF-YA might be functionally related to adipogenesis, we lentivirally transduced BM-MSCs with NF-YA shRNA (shNF-YA) and subsequently induced differentiation. During adipogenesis (d14) NF-YA mRNA levels in shNF-YA BM-MSCs were constantly reduced to 51.9% of the respective levels in control cells. A 31.3%, 70.1% and 74.3% reduction was also observed in PPARG, CEBPA and LPL levels, respectively, in shRNA transduced cells compared to controls. Finally, cytochemical Oil-red O staining revealed a lack of lipid droplets in shRNA transduced cells. **Summary and Conclusions.** We have shown for the first time that NF-YA mRNA and protein levels increase during BM-MSC adipogenic differentiation and that NF-YA transcription levels strongly correlate with the adipocytic-related genes CEBPA, PPARG, and LPL. These findings are suggestive of a novel role of NF-YA in adipogenesis, a notion which is further corroborated by the decreased differentiation following NF-YA knock-down. We also observed a moderate decrease of NF-YA mRNA levels after long *in vitro* BM-MSC expansion. Whether this finding is related to BM-MSC loss of stemness and/or senescence is under investigation.

0711

PATIENTS WITH GAUCHER DISEASE EXHIBIT INCREASED FREQUENCIES OF PERIPHERAL BLOOD ACTIVATED LYMPHOCYTES AND SEVERELY IMPAIRED REGULATORY T-CELLS

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Background. Gaucher disease (GD) is a Lysosomal Storage Disorder, characterized by splenomegaly, bone disease and peripheral blood cytopenias, resulting from the accumulation of undigested lipid-replete macrophages in the bone marrow and the reticuloendothelial system. Patients with GD commonly exhibit a chronic inflammatory response, as yet not completely understood. We have previously shown that these patients also exhibit constitutively deceased numbers of peripheral blood absolute lymphocyte count and of CD3+, CD4+ and CD4+/HLA-DR+ T-lymphocytes, as well as increased percentages and absolute numbers of CD8+/CD45RO+, CD8+/HLA-DR+ T-lymphocytes. **Aims.** To investigate the frequencies of regulatory T-cells (T-regs) and the polarization of T-helper cells in the same cohort of patients. **Patients and Methods.** A three color whole blood method of flow cytometry was used for evaluation of T-lymphocyte subpopulations, during the routine work-up of 17 patients with type I GD, after obtaining informed consent. T-regs were evaluated as the fraction of CD4+/CD25^{high}+/FOXP3+ cells. Cytoplasmic expression of IL-2, IL-4, IFN-gamma, IL-10 and IL-13 was evaluated after short activation with PMA, ionomycin and blockage of Golgi apparatus with BFA.Results were compared with those of 21 normal controls, sex-and age-matched. All estimations were performed in the absence of any infection or other inflammatory condition. **Results.** Compared to controls, we have again confirmed that patients with GD exhibited significantly decreased absolute lymphocyte count (x±SEM: 1738±235 versus 2053±157/μl, p=0.039), CD3+ lymphocytes (1197±128 versus 1403±139/μl, p=0.031) and CD3+/CD4+helper T-lymphocyte count (38.8±3.3% versus 44.7±3.6%, p=0.022, or 676±88 versus 931±101/μl, p=0.004). Conversely, they exhibited increased proportion of CD3+/CD8+ suppressor T-cells (26.5±2.4% versus 19.2±2.1%, p=0.007, or 446±52 versus 402±39/μl, p= 0.199) resulting in a significant reduction of the CD4/CD8 ratio (1.53±0.19 vs 2.26±0.22, p=0.0012). Patients with GD exhibited also increased proportions of activated CD4+/CD25+ lymphocytes (2.54±0.77, versus 2.04±0.94%, p=0.062), CD4+/HLA-DR+ lymphocytes (2.83±0.46 versus 1.47±0.27%, p=0.0021) and CD8+/HLA-DR+ lymphocytes (1.07±0.18 versus 0.47±0.06%, p<0.0001). Moreover, patients with GD had significantly lower percentage of CD4+CD25^{high}-FOXP3+ T-regs (1.27±0.22 versus 2.09±0.41%, p=0.017), and among CD4+/CD25^{high}+ cells, significantly lower percentage of FOXP3+ cells (76.1±5.5 versus 85.6±5.9%, p=0.004). Lymphocytes of patients with GD exhibited a clear TH1 polarization (CD4+IFNgamma$_{cy}$ unstimulated: 1.23±0.41 versus 0.58±0.14%, p=0.077 and following stimulation: 38.8±3.1 versus 23.6±2.3%, p=0.0003, CD4+IL2$_{cy}$ unstimulated: 0.30±0.06 versus 0.13±0.03, p=0.009 and following stimulation: 65.8±4.0 versus 62.5±3.1%, p=0.396, CD4+IL13$_{cy}$ unstimulated: 0.104±0.026 versus 0.072±0.020, p=0.170, and following stimulation: 0.549±0.085 versus 0.404±0.064, p=0.094, CD8+IFNgamma$_{cy}$ unstimulated: 0.51±0.34 versus 0.23±0.05%, p=0.211, and following stimulation: 62.1±7.3 versus 43.9±4.8%, p=0.025). Finally both, the percentage and the absolute number of CD5+/CD20+ autoreactive B-lymphocytes were not significantly different between patients and controls (2.45±0.58 versus 1.94±0.37%, and 60±18 versus 47±14/μl, p: n.s. for both comparisons).*Discussion and* **Conclusions.** Taken together, our results demonstrate that in patients with GD there is a significant numerical impairment of T-helper lymphocytes and a constitutive activation in both, the CD4+ and the CD8+ cell compartment, associated with a significant decrease of T-regs, although the autoreative B-lymphocytes are not always found increased. These findings may explain the chronic inflammatory reaction, as well as the increased incidence of lymphoid malignancies, which has been reported among patients suffering from GD.

0712

CHARACTERIZATION OF HEMATOPOIETIC STEM AND PROGENITOR CELL ADHESION TO SURROGATE MICROENVIRONMENTS

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Background. Hematopoietic stem cells reside in specialized microenvironments - stem cell niches - consisting of different cell types and extracellular matrices. Within these niches, a multitude of signals influence particular stem cell behaviours such as quiescence, self-renewal and differentiation. The dysregulation of these niches, either in structure or function, affects hematopoiesis and has been implicated in disease states of the bone marrow such as leukemia or myelodysplastic syndromes (MDS). Leukemic stem cells have been shown to co-opt cues from the microenvironment to further leukemic progression and mitigate the effects of cytotoxic therapies. Amongst the large number of signals in the niche, there are extracellular matrix components not only produced by the surrounding niche cells but also by the hematopoietic stem cells (HSCs) themselves. Understanding the complexity of stem cell-niche interactions could help to elucidate novel aspects that might lead to new targeted therapies. **Aims.** Given the requirements of leukemic cell interaction with the niche, our goal was to understand the interactions of healthy hematopoietic stem and progenitor cells (HSPCs) with defined extracellular matrix (ECM) components in an *in vitro* setting. We used cell lines as well as normal bone marrow CD34$^+$ cells to investigate adhesion to various substrates found in the bone marrow microenvironment. **Methods.** Using high-throughput microscopy, we developed a novel and robust adhesion assay to study the frequently limited cell numbers of HSPC obtained in certain diseases such as MDS. We combined this with multiparameter flow cytometry in order to study the various subsets of normal CD34$^+$ HSPCs. **Results.** Over 90% of healthy CD34$^+$ cells adhered to the well-studied substrates such as fibronectin and vascular cell-adhesion molecule 1 (VCAM-1); 37% of CD34$^+$ cells adhered to laminin and osteopontin and only 15% to collagen-I, suggesting heterogeneity within the adhesion of different HSPC-subsets. Overall, CD38$^-$ cells adhered more than CD38$^+$ to all the substrates studied, demonstrating the differential adhesion of primitive and mature progenitors. Regarding committed myeloid progenitors, megakaryocyte-erythroid progenitors (MEP) were the most adherent population, strongly attaching to all the substrates studied. Similar numbers of common myeloid progenitors (CMP) and granulocyte-macrophage progenitors (GMP) were recovered in the majority of the adherent vs. non-adherent fractions. Interestingly, both progenitor populations were enriched in the non-adherent fraction of VCAM-1 wells, suggesting that CMPs and GMPs do not bind to VCAM-1. Amongst the substrates studied, HSCs preferentially adhered to laminin and fibronectin; the number of adherent cells increased 5-fold and 2-fold in the laminin and in the fibronectin groups respectively. **Summary and Conclusions.** Herein, we

demonstrated the inherent adhesion heterogeneity of normal CD34+ progenitors towards defined ECM. These differences in adhesion are likely to play an important role in normal hematopoietic development as well as in malignant transformations. Further studies on HSC and their attachment towards the ECM could provide a sensitive tool for targeted therapy in hematological diseases.

0713

ROLE OF DNA DEMETHYLATION AGENT 5-AZA DEOXYCYTIDINE IN THE PRODUCTION OF VON WILLEBRAND FACTOR IN HUMAN BONE MARROW DERIVED CELLS CULTURED IN 3D PELLETS

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Epigenetic regulation of gene expression is recognized as a key mechanism governing cell determination, commitment, and differentiation as well as maintenance of cell state. An important component of epigenetic control is DNA methylation, which is associated with gene silencing. 5-aza deoxycytidine (5-aza-dC) is a cytidine analogue that traps the DNA methytransferase and consequently inhibits the methylation of the newly synthesized DNA strand in dividing cells. We have previously shown the potential to enhance human bone marrow stromal cell (HBMSC) differentiation along the osteogenic lineage with 5-Aza-dC in monolayer as well as in pellet culture. Pellets were formed with 5 X 10^5 cells and cultured in plastic tubes. When HBMSCs were seeded on glass tubes, the cells formed, initially, a monolayer that subsequently formed into a sphere and resided on migration at the air-media interface. These self assembled pellets were 16 times larger than their comparable pellets cultured in the classical plastic tubes. Untreated pellets displayed distinct collagen I matrix formation corresponding to osteogenic matrix. Pellets formed with HBMSCs pretreated with 5-Aza-dC displayed significantly less collagen matrix and extensive von-Willebrand factor (vWF) expression. Extensive vWF immunostaining was observed in the pellets in particular within the centre of the pellet with what appeared a loose capillary network. This study demonstrates the induction of vWF in HBMSCs by 5-Aza-dC although the exact cell phenotype remains unclear. These HBMSC 3D pellets expressing vWF protein offer an interesting model to examine tissue development and factor screen/analysis on the production of vWF and cell differentiation. Although the precise mechanism of 5-Aza-dC induction in this process is unclear, these studies emphasis the importance f DNA methylation in cell function and the potential to harness the activity of epigenetic modifier in cell lineage determination and function.

0714

CONGENITAL MALFORMATIONS IN PATIENTS OF THE TUNISIAN FANCONI ANEMIA REGISTRY

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Background. Fanconi anemia (FA) is an autosomal recessive disorder characterized by congenital malformations, bone marrow failure and increased risk of development of leukemia and other malignancies. Birth defects are heterogeneous, affecting diverse systems of the body and especially the skin, the bones and the urinary tract. Thus, the patients display a wide variety of phenotype. Here, we report the frequencies of congenital abnormalities in FA patients of the Tunisian Fanconi Anemia Registry (TFAR). **Aims.** To analyse different malformations in FA patients in Tunisia. **Methods.** This study concerns the 174 patients included in the TFAR, from January 1984 until November 2011. We report frequencies of different congenital malformations in our FA patients. **Results.** Growth retardation was seen in 109 patients (62%). Skin abnormalities were present in 139 patients (80%): café-au-lait spots (64.3%), hyperpigmentation (51.1%), hypopigmentation (12%). Head was affected (microcephaly, microphtalmy, fine lines, triangular facies) in 137 patients (78%). Explorations of the urogenital system were done in only 55% of our patients. They detected anomalies in 80% of explored cases: ectopic kidney (41patients), single kidney (9 patients), horseshoe kidney (6 patients), ectopic testicle (7 patients), urinary tract (10 patients). 47% of our patients had bone abnormalities, affecting the thumb (30%), the other fingers and toes (15%). Congenital dislocation of the hip was found in 5 patients and spina bifida in one patient. Neurologic system involvement was seen in 4%, with mental retardation in 3%, hearing loss in 1%, strabism in 1%. Gastrointestinal system anomalies were reported in 2.8%. Endocrinopathies have concerned 2.4% of our FA patients. **Conclusions.** This retrospective study is one of the largest series of FA patients in the literature. Globally, the frequency of different malformations, here, approach that described in several studies. However, some congenital anomalies, reported in other series but not in our one, such as cardiac defects, must be more carefully searched by systematic exams. Recent standardization of FA patient support in Tunisia may help better analysis of these findings.

Chronic lymphocytic leukemia - Biology 2

0715

GENOMIC COPY NUMBER ALTERATIONS IN HIGH-RISK CLL PATIENTS HARBORING TP53 DEFECTS: INSIGHTS FROM CONSECUTIVE INVESTIGATIONS

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Background. In chronic lymphocytic leukemia (CLL), recurrent copy number alterations (CNAs) have been described (deletions of 13q14, 17p13 and 11q22, and trisomy 12), that are typically present in variable proportions of leukemic cells. Significantly, genomic lesions can accumulate during the disease course, which is evident when pre-treatment and relapse samples are compared. Among them, *TP53* defects (mutations/deletions), that are in general associated with genomic instability, are selected in approximately one-fifth of treated CLL patients with an originally intact gene. Taken together, these phenomena likely contribute to the chronic relapsing course of CLL and the development of chemotherapy resistance. **Aims.** Our study aimed to describe specific CNAs accompanying selection of *TP53* defects by therapy in CLL patients, and compare them to those present in patients having either wild-type (wt) *TP53*, or mutant (mut) *TP53* in all subsequent samples. **Methods.** Consecutive peripheral blood samples from 22 well characterized CLL patients (unmutated IGHV in 18/22 cases) were collected. Since therapy administration can facilitate expansion of more aggressive subclones in the disease relapse, samples separated by therapy were preferentially selected for the study. *TP53* mutations were identified by FASAY and direct sequencing. Genomic DNA (gDNA) was isolated from separated CD19+ or mononuclear cells and analyzed using Cytogenetics 2.7M Array (Affymetrix). Further data parsing, filtering, and mining were performed with in-house computer programming. Array results were compared to results of I-FISH and metaphase cytogenetics with CpG/IL-2 stimulation. **Results.** In total, 46 gDNA samples were analyzed (two or three consecutive samples in 20/22 and 2/22 patients, respectively). Median interval between the samplings was 24 months (range 9-55 months). Altogether, 18 wt-*TP53* and 28 mut-*TP53* samples were analyzed. Concerning a simple number of CNAs, no significant differences between wt- and mut-*TP53* samples were observed. It might be explained by the fact that majority of patients belonged to the high-risk CLL and, more importantly, that accumulation, but also elimination of CNAs was observed during the disease course. The original and follow-up *TP53* status was used to divide the patients into three groups: group A with wt-*TP53* in consecutive samples (3/22 patients), group B with mut-*TP53* in consecutive samples (8/22), and group C with selection of new *TP53* mutations after CLL therapy (11/22). Group C exhibited the most dramatic genomic changes. Specifically, del(17p) leading to complete inactivation of *TP53*, as well as del(4p), del(4q), del(6q), and del(13q) (including *miR-15-1/16a* and *RB1* genes) occurred frequently in this group. Interestingly, in this group we also noted recurrent vanishing of del(11q); the subclone was likely replaced by the one harboring *TP53* defect. In group B, only del(15q) appeared recurrently. **Summary**. Our gradual analysis catalogues the appearance, but also disapperance of recurrent CNAs during the disease course of CLL. In particular, selection of *TP53* defects after therapy seems to be accompanied by accumulation of specific CNAs in our patients. We assume that these CNAs potentially drive disease aggressiveness, whereas CNAs which appear as well as diminish likely do not play role in disease progression. *Supported by CZ.1.05/1.1.00/02.0068, CZ.1.07/2.3.00/20.0045, CZ.1.07/2.4.00/17.0042, MSM0021622430, MUNI/A/0784/2011, GACR204/09/H058.*

0716

EARLY DETECTION OF MINIMAL RESIDUAL DISEASE DETECTION TARGETING CD160 IN CHRONIC LYMPHOCYTIC LEUKAEMIA PREDICTS PROGRESSION FREE SURVIVAL AND CORRELATES WITH BIOLOGICAL PROGNOSTIC RISK GROUPS

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Background. The prognosis of patients diagnosed with chronic lymphocytic leukaemia (CLL) is highly variable. Prognostication is currently based on a number of clinical and biological factors. Minimal residual disease (MRD) detection during and post-therapy is emerging as a powerful predictor of outcome. **Aims.** This study utilizes a multi-parameter flow cytometric assay to evaluate the time toMRD detection and correlate this with established prognostic markers and event free survival (EFS). **Methods.** Utilizing the anti-CD160 monoclonal antibody (BY55, Coulter Immunotech, Marseille, France), we have developed a single tube, highly sensitive flow cytometric assay (CD160FCA) incorporating a sequential gating strategy. 97 patients were investigated for MRD and response rates and EFS (time to next therapy, intervention or death) were determined. The time to MRD detection post therapy was correlated with the prognostic indicators, ZAP-70, CD38, beta-2 microglobulin (b_2M) and cytogenetic abnormalities. **Results.** CD160FCA could be used to monitor patients for MRD, including post-immunotherapy with Rituximab and Campath. 220 samples were analysed. Of patients achieving a complete remission (CR) by current NCI guidelines, those with residual disease by CD160FCA (CR-CD160+) had significantly shorter EFS compared with CR-CD160 negative patients (532 days *vs* 1825 days, P<0.0001). Interestingly, those patients who achieved a partial remission (PR), but were MRD positive, had a shorter EFS versus PR-CD160 negative patients (365 days *vs* 669 days, P=0.039).Patients with known adverse risk factors had a significantly shorter time to disease detection (TTD) by CD160FCA: high b_2M (184 days *vs* B_2M normal 852 days), CD38 positivity (185 days *vs* 638 days) and ZAP-70 positivity (276 days *vs* 760 days). Patients with poor risk cytogenetics (17pdel: 90 days, 11qdel: 122 days) had significantly shorter TTD over those with intermediate cytogenetics (+12: 244 days) and those with good risk (13qdel: 761 days). Of interest those patients with biallelic 13q- had a significantly shorter TTD (151.5 days). **Conclusions.** Detection ofMRD using CD160FCA, a single tube, tumour specific multi-parameter flow cytomteric assay, can independently predictEFS irrespective of therapy given and clinical outcome stratification (CR or PR). Patients with adverse prognostic markers had a significantly shorter TTD over those with favourable ones. Detection ofMRD by CD160FCA offers a simple biomarker endpoint as a surrogate for response assessment in clinical trials and the intriguing possibility of targeting CD160 forMRD eradication using therapeutic anti-CD160 chimaeric antibodies under development.

0717

THE ABSOLUTE NUMBER OF REGULATORY T-CELLS AT DIAGNOSIS CAN PREDICT A SHORTER TIME TO THE FIRST TREATMENT IN PATIENTS WITH RAI STAGE 0 CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Regulatory T cells (Tregs) are increased in chronic lymphocytic leukemia (CLL) and correlate with clinical and biological features of active/progressive disease. However, little is known about a possible relationship between Tregs and the time to first treatment (TFT) in patients with early stage CLL. **Aims.** To investigate this specific topic, we conducted a prospective multicenter study in patients with Rai stage 0 CLL, in whom the absolute number of Tregs was determined at the time of diagnosis and correlated to main clinical and biological prognostic factors, as well as to the need of receiving any specific therapy for CLL during the course of the disease. **Methods.** Seventy-five consecutive and previously untreated patients with Rai stage 0 CLL (mean age 68 years; range 44-90 years; M/F ratio 1.2) were evaluated. Circulating Tregs were determined at the time of diagnosis by means of multidimensional flow cytometry (CD4-PerCP/CD25-PE-Cy7/CD127-PE/CD45-APC-Cy7). Tregs

were defined by the expression of CD4, CD25 at high density and CD127 at low density or undetectable levels. The absolute Treg cell count was calculated by multiplying their percentage value (of all lymphocytes) by the absolute lymphocyte number as provided by the hematology analyzer (dual platform approach). **Results.** After a median follow-up of 30 months, twelve patients (16%) needed therapy at some time from the diagnosis, according to IWCLL criteria (median time: 16 months; range 1-34 months). At baseline, age, gender, and LDH serum levels, as well as ZAP-70 and CD38 expression, were not statistically different between untreated and treated patient groups. By contrast, treated patients showed a higher number of total peripheral white blood cells and B-lymphocytes (p <0.001), lower hemoglobin values (p 0.015), higher platelet count (p 0.012), a greater number of cases with unmutated IgVH status (p 0.025) and a higher frequency of high risk cytogenetic abnormalities (p 0.05) than patients who did not need therapy. Of interest, a greater median number of circulating Tregs (n. 79, range 35 - 112) was detected at diagnosis in treated patients, with respect to patients who did not receive therapy during the observation period (n. 30, range 5 - 54) (p<0.001). Multivariate analysis confirmed that the absolute number of Tregs was the only independent predictor of TFT in these patients (p<0.001), the best predictive cut-off (ROC analysis) being 41/µL. **Conclusions.** Our data show that an increased absolute number of Tregs found at diagnosis may be predictive for a shorter TFT in low risk, Rai stage 0 CLL patients.

0718

ANALYSIS OF SERUM CHEMOKINES AND CYTOKINES IN BINET STAGE A CLL: SIL2RALPHA IS AN INDEPENDENT PREDICTOR OF PROGRESSION FREE SURVIVAL (PFS)

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Background. Chemokines and cytokines are an integral part of the CLL specific tumor microenvironment. The exact biological role of cytokines and chemokines needs to be defined, especially in the light of distinct prognostic and biological subgroups of patients. **Aims.** To characterize chemokines and cytokines of the CLL microenvironment in more detail and to evaluate their impact on prognosis. **Methods.** Serum levels of chemokines and cytokines in a prospective cohort of 157 previously untreated Binet stage A patients (pts) from the CLL1 trial of the German CLL Study Group (GCLLSG) were measured. Serum samples had been centrally collected at study entry and stored at -80°C and were analyzed on a luminex-based multiplex platform, allowing simultaneous screening of 27 chemokines and cytokines. Median survival time was 83 months. For all serum parameters univariate and multivariate analyses were performed for progression-free and overall survival. Association with established risk parameters, like high leukocyte counts, FISH cytogenetics, IgHV status was evaluated. In addition, hierarchical clustering was used to identify sets of cytokines whose expression levels correlated among individual patients. **Results.** High CCL3 levels strongly correlated with high CCL4 levels (p<0.001) and tended to be associated with unmutated IgHV status (p=0.06), supporting the role of BCR triggering in biologically selected CLL pts. Serum levels of CCL2 and CCL17, both binding chemokine receptor CCR4, were concordantly elevated compared to healthy controls (p<0.001), suggesting a possible role of chemoattraction of CCR4+ T cells towards these chemokines in CLL. Pts with high serum levels of CCL21 had higher levels of CXCL10 (p<0.001) and CXCL12 (p=0.02). Elevated VEGF levels were strongly associated with elevated EGF level (p<0.001). High VEGF levels correlated with high white blood cell counts (p=0.008) and showed a trend towards association with del(11q) (p=0.07). Hierarchical clustering analysis divided the patient cohort into two distinct subgroups. Serum levels of EGF, TNFalpha, VEGF, CCL2, CCL3, CCL4, CCL21, and Il16 were significantly higher in Cluster 1 as compared to Cluster 2 (p<0.003, adapted for multiple testing). There was no difference in progression-free survival and overall survival between the two groups.High sIl2Ralpha levels were associated with decreased PFS and OS. These results could be confirmed by conventional ELISA. Mean estimated OS survival in pts within the highest quartile of sIl2Ralpha levels was 98 months compared to 111 months in the lower three quartiles (p=0.002). For PFS, but not for OS sIl2Ralpha could be identified as an independent prognostic factor. (HR 2.5, 95% CI 1.4-4.8, p=0.004). **Summary and Conclusions.** Patients in Binet stage A CLL can be classified into two subgroups with distinct chemokine and cytokine expression profiles. Median serum levels of different chemokines correlated with distinct biological characteristics of CLL patients, like genetic abnormalities or clinical parameters. sIl2Ralpha could be identified as an independent prognostic variable for PFS in early CLL.

0719

CLONAL DIVERSITY OF THE T CELL REPERTOIRE IN CHRONIC LYMPHOCYTIC LEUKAEMIA ASSOCIATES WITH STEREOTYPY PREDICTS DISEASE PROGRESSION

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Introduction. Antigenic selection in chronic lymphocytic leukaemia (CLL) is evidenced by a skewed BCR repertoire and continuing BCR signaling detected in subgroups of CLL. Evidence for a possible T cell involvement in maintaining the clone includes severe skewing of T cell subsets and the involvement of CD4 cells in so-called “proliferation centers”in lymph nodes. However, to date clonal or immunologic identities of these T cells are unclear. In Tcl1 transgenic mice we recently showed that CLL clones can drive changes in the T cell repertoire. Significant changes in subset distribution and clonal selection of T cells was apparent in murine CLL. **Methods.** We aimed to establish evidence for clonal T cell skewing in peripheral blood from human CLL. In 53 previously-untreated patients were characterized regarding BCR V gene usage, mutation status and clinical parameters. TCR V beta clonality in sorted CD4 cells was analysed by CDR3 length polymorphism in 20 TCR genes and by TCR V gene-specific flow cytometry in a subset. **Results.** BCR analysis showed that 10 IgVH V-genes accounted for > 75% of the cases. Stereotyped CDR3 regions were present in 41% of cases and 25% of the patients showed unmutatetd VH gene sequence. TCR CDR3 size distribution patterns showed heterogeneity of monoclonal CD4 T cell patterns between patients. While about half of the patients showed polyclonal patterns in all their TCR CDR3 regions, the others showed single or multiple clonal TCR families. Corresponding flow cytometric analyses showed sizeable T cell clones between 5 and 48% of total CD4 cells. In patients with longitudinal samples relevant stability of TCR patterns could be observed over time. As a control, TCR clonalities were not significantly associated with CMV serostatus in patients.Next we compared the TCR clonality database with established BCR characteristics. No strong association between mutation stae and clonality was observed. Overall we found no strong association with any specific IgVH gene, but intriguingly, we found a significant difference in the clonal T cell frequencies between stereotyped and non-stereotyped CLL. This difference was very prominent looking at the unmutated cases only (p<0.001), with unmutated stereotyped CLL displaying practically no T cell clonalities. Since stereotyped CLL has been speculated to be a B1 cell equivalent and murine B1 cells are prototypically T cell independent, our finding may suggest a different immunologic network driving stereotyped and non-stereotyped CLL. Functional studies to test the effects this difference *in vitro* are under way. Finally, analysed the influence of T cell clonality on clinical behaviour of CLL disease. The presence of more than one clonal TCR family was a significant predictor of a short treatment-free interval (p=0.03). This was true for both, patients with mutated and unmutated IgVH receptors, although it remained a trend in the latter. **Conclusions.** Our results suggest that a restricted CD4 T cell diversity could be important for CLL progression and that there are differences in the recruitment of clonal T cells between CLL of stereotyped and non-stereotyped origin.

0720

IS THERE A ROLE FOR BONE MARROW BIOPSY EXAMINATION IN THE DIAGNOSTIC WORK-UP OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA? A REAPPRAISAL IN THE ERA OF BIOLOGICAL PROGNOSTIC MARKERS

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According to the iwCLL/NIH guidelines for the diagnosis and treatment of CLL, bone marrow biopsy (BMB) is no longer considered as mandatory at

diagnosis and only recommended for the evaluation of cytopenias of unknown cause, before the initiation of treatment and for assessing response after treatment for patients enrolled in clinical trials. Though practical, this approach hinders the understanding of tumor dynamics within the compartment where the clonal B cell population may arise. Herein, we present the results from the multidisciplinary examination of diagnostic BMB samples from a series of 159 unselected CLL cases. The patient group included 96 males and 63 females with a median age of 64 years (range, 26-84). Most CLL patients were at early clinical stages (only 36/159 cases in Binet stages B/C). Our analysis confirms and significantly extends previous observations regarding the favorable impact of the nodular pattern of neoplastic lymphocytic infiltration (NLI) of the BM. In particular, it indicates a biological sub-context by showing that a nodular infiltrative component is significantly associated with mutated IGHV genes. In the vast majority of cases, the neoplastic population consisted almost exclusively of small, "well-differentiated"lymphocytes. Medium- or large-sized cells were admixed in 4 and 1 cases, respectively. Finally, 8 and 3 cases exhibited lymphoplasmocytoid and plasmacytic differentiation, respectively, as confirmed immunohistochemically by cytoplasmic monoclonal immunoglobulin of the same isotype as the bulk of the NLI. Interestingly, 34/159 cases (21%) showed a NLI of less than 30%. Though the possibility of sampling-related issues cannot be a priory discredited, this finding might reflect a focal pattern of BM infiltration or, alternatively, a distinctive compartmentalization of the neoplastic clone. In keeping with previous reports, the extent of BM involvement was also found to be prognostically relevant, since cases with less than 30% NLI of the BM exhibited longer time-to-first treatment, independently of IGHV gene mutational status. Regarding the hematopoietic marrow, 82 cases showed hyperplastic granulocytic series with a shift to the left and, occasionally, concomitant dysplastic changes of the mature forms. Hyperplasia of the erythroid and megakaryocytic series largely in a context of normal or decreased hemoglobin levels and platelet counts was observed in 97/157 and 121/158 cases, respectively. Most cases with erythroid and/or megakaryocytic hyperplasia exhibited dyserythropoiesis and dysmegakaryopoiesis. In such cases, CD34+ cell counts were always below 2%. In conclusion, we confirm that BMB examination permits identification of the rare CLL subtypes with lymphoplasmocytoid or plasmacytic differentiation. More importantly, on the evidence presented here, BMB examination offers important information not only for diagnostic purposes but also for elucidating CLL pathobiology, e.g. by pointing to CLL-associated hematopoietic autoimmunity even in patients with no cytopenias at diagnosis. By logical extension, it is expected to contribute to unraveling the mode of action of novel therapies which target the interaction of CLL cells and their (micro)environment.

0721

THE CLINICALLY ACTIVE BTK INHIBITOR PCI-32765 TARGETS B-CELL RECEPTOR- AND CHEMOKINE-CONTROLLED ADHESION AND MIGRATION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Several novel small molecule drugs targeting the B-cell antigen receptor (BCR) signalosome have recently demonstrated promising efficacy in the treatment of B-cell non-Hodgkin lymphoma. Most of these agents, including the BTK inhibitor PCI-32765, display an unexpected clinical response in chronic lymphocytic leukaemia (CLL): a rapid and sustained reduction of lymphadenopathy accompanied by transient lymphocytosis, which is reversible upon temporary deprivation of the drug. We have previously demonstrated that the B-cell antigen receptor (BCR) controls integrin $\alpha_4\beta_1$-mediated adhesion of B cells to fibronectin and VCAM1, which is mediated by Bruton's tyrosine kinase (BTK). More recently, we have shown that also chemokines induce activation of BTK and that integrin-mediated adhesion and migration in response to CXCL12 or CXCL13, as well as *in vivo* homing to lymphoid organs, is impaired in BTK-deficient B cells. **Aims.** We hypothesized that the clinical response observed upon PCI-32765 treatment reflects attenuated lymph node and bone marrow homing and retention of the CLL cells, due to impaired BCR- or chemokine-controlled integrin-mediated adhesion or migration. **Methods.** The Burkitt lymphoma cell line Namalwa or primary patient CLL cells, pretreated with PCI-32765, were allowed to adhere to either fibronectin- or VCAM-1-coated surfaces in the presence of anti-IgM, PMA, or chemokines (CXCL12, CXCL13 or CCL19), or were allowed to migrate towards these chemokines in VCAM-1-coated Transwells, **Results.** We found that PCI-32765 strongly inhibits BCR-controlled signaling and integrin $\alpha_4\beta_1$-mediated adhesion to fibronectin and VCAM-1, both in Namalwa and primary CLL cells. Furthermore, PCI-32765 also inhibits CXCL12-induced signaling and CXCL12-, CXCL13- and CCL19-induced adhesion and migration of primary CLL cells. **Conclusions.** Our data indicate that inhibition of BTK by PCI-32765 overcomes BCR- and chemokine-controlled homing and retention of the malignant B cells in their growth- and survival-supporting microenvironment, resulting in clinically evident CLL regression (Figure 1).

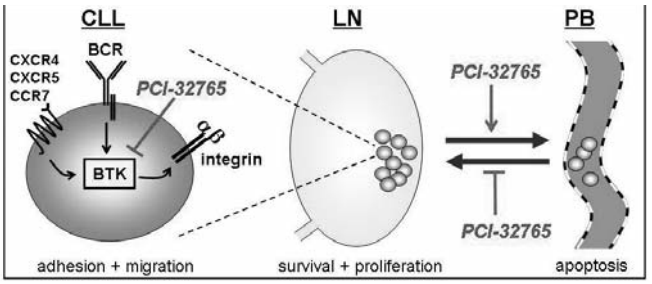

Figure 1. Inhibition of BTK impairs BCR-controlled integrin-mediated adhesion and chemokine-induced adhesion and migration of CLL cells.

0722

MAPPING THE TARGETS OF DASATINIB IN CHRONIC LYMPHOCYTIC LEUKEMIA; DISTINCT ROLES FOR ABL AND BTK IN DRUG RESISTANCE AND ADHESION/MIGRATION

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Background and Aims. Chronic lymphocytic leukemia cells in the protective lymph node (LN) microenvironment are chemoresistant due to upregulation of Bcl-XL, Mcl-1 and Bfl-1. In vitro, this can be mimicked via CD40-stimulation of CLL cells, which affords resistance to various chemotherapeutics. Novel drugs that target kinases involved in B cell signaling are currently in clinical development for CLL. We showed previously that Dasatinib, a broad spectrum inhibitor that targets Abl- and Src-family kinases, prevented CD40-mediated anti-apoptotic changes in CLL, and restored drug sensitivity. Here, we coupled Dasatinib to an affinity matrix and characterized its spectrum of targets in CLL. **Methods and Results.** Using pull-down, Abl and Btk were identified as specific targets of Dasatinib upon CD40 triggering. Functionally, Abl was mainly responsible for conferring drug resistance, while Btk mediated adhesion/migration to attach CLL cells in the LN. Additional kinases were identified by mass spectrometry and included 8 known targets of Dasatinib (e.g. Lyn, Fyn, Yes) and 7 CLL-specific kinases. These will be studied further for their contribution to drug resistance, apoptosis and adhesion/migration. **Conclusions.** By mapping the contribution of all kinases targeted by Dasatinib, this study highlights the crosstalk between BCR and CD40 pathways in CLL and may result in new therapeutic strategies.

0723

COMPARATIVE GENE EXPRESSION ANALYSIS PROVIDES NOVEL THERAPEUTIC TARGETS IN CLL

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Background. The different clinical and biological prognostic markers validated are not capable of explaining the pathogenesis of the CLL and the implication in the development of the disease is still under investigation. The identification at the molecular level of new variables thanks to massive analysis of gene expression profiles can contribute to a better knowledge of the pathogenesis of the disease, to the development of new prognostic factors, fit the treatment on the basis of the specific risk and the identification of new therapeutic targets. **Aims.** Identify a molecular signature which lead to find new therapeutic targets in CLL. **Methods.** Thirty-eight CLL lymph node samples were analysed and compared with normal lymph nodes in order to find differential gene expression profiles signatures. cRNA hybridization was performed in Agilent 44K microarrays. The arrays were scanned with a G2565BA microarray scanner system. After data normalization and preprocessing, data were

analysed with bioinformatic tools provided by Babelomics. Self-organising tree algorithm (SOTA) was use for hieralchical clustering. Clinical and biological markers were obtained from patients enrolled in the study. **Results.** Two molecular subtypes were identified, with 1092 genes with differential expression between both groups, with a fdr<0,05. After Gene Set Enrichment Analysis (GSEA), 4 molecular parhways were enriched in group A and 1 molecular pathway in group B, with a FDR q value <0,25. Group A pathways were IL12, ILR1, IL5, TALL1 and Group B pathways were CDK5. No difference in Treatment-Free, Progression or Overall Survival were observed between both group of patients. When compared CLL samples with reactive lymph nodes, 1178 genes showed differential expression pattern with a fdr<0,05. After Gene Set Enrichment Analysis, BCR molecular parhways was the only pathway with enrichment in CLL samples, whereas Germinal Genter and Stromal Germinal Center were enriched in normal lymph nodes, with a FDR q value <0,25. **Conclusions.** Despite molecular heterogeneity of CLL, we have not found a prognostic molecular signature. However, we have observed different molecular profiles which may lead to find therapeutic target in subgroups of patients and receive a personalised medicine.

0724

HDAC ISOENZYME EXPRESSION IS DEREGULATED IN CHRONIC LYMPHOCYTIC LEUKEMIA B-CELLS AND HAS A PROGNOSTIC SIGNIFICANCE

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Background. Increasing evidences have highlighted the role of epigenetic modifications such as histone acetylation in the development of human cancer. Indeed, histone deacetylases (HDACs) including Sirtuins (SIRT) play an important role in transcriptional regulation by changing the chromatin structure and their deregulation has been reported in various cancers including hematological malignancies. **Aims**. In this context, we performed a complete and comprehensive study of the 18 HDACs (HDAC1 to 11 and SIRT 1 to 7) isoenzyme expression in Chronic Lymphocytic Leukemia (CLL) B-cells. **Methods.** In the present study, HDAC expression was quantified by real-time PCR on RNA extracted from purified CD19+ cells in a cohort of 200 CLL patients with a median follow up of 74 months and compared to that of peripheral blood B cells obtained from 20 age-matched healthy volunteers. The expression of the 18 HDACs was thereafter correlated to various classical prognostic factors and to treatment and survival data. **Results.** Comparison with peripheral normal B cells showed that HDAC2 (fold: -1.75) and SIRT4 (-2.78) were statistically downregulated while HDAC6 (+1.83), HDAC7 (+2.80), HDAC11 (+2.18), SIRT3 (+1.54), SIRT6 (+1.31), and SIRT7 (+1.43) were upregulated in CLL samples (P<0.05). When we compared the expression of all HDACs in different prognostic subgroups based on well-known prognostic factors (IgVH status, ZAP70, LPL, CDC38), no differential expression was found. Taken individually HDAC6 was significantly correlated with treatment-free survival (TFS) and HDAC3, SIRT2, 3 and 6 with overall survival (OS) but the P_{value} has a borderline significance. However, a multivariate Cox regression stepwise analysis indicated that HDAC6, 7, 10 and SIRT3 were independent prognostic factors (hazard ratio - HR - ranging from 0.39 to 1.95). Therefore, these factors were combined in a HDAC score (from 1 to 4 poor prognostic markers) that allows best treatment risk stratification: patients with a score of 1-2, 3 and 4 had a median TFS of 107, 57 and 26 months respectively (HR=4.23, P<0.0001). For OS, only SIRT2 is an independent predictor dividing patient in 2 groups with median OS of >360 and 237 (HR=0.39, P=0.0110).

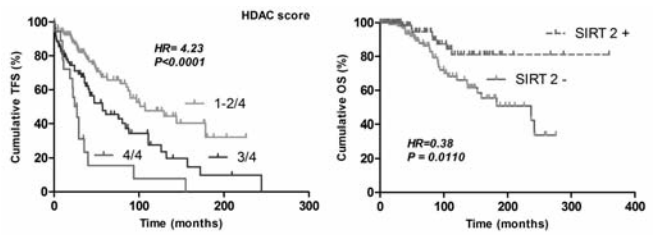

Figure 1. Prognostic significance of HDACs.

Conclusions. HDAC expression is clearly deregulated in CLL cells when compared to normal controls but no correlation between HDAC expression and classical prognostic factors was observed. However, a score combining HDAC6, 7, 10 and SIRT3 is a strong TFS predictor indicating that more than one HDAC had influence on the need of treatment while only SIRT2 is a significant predictor of OS. Interestingly, poor prognosis could be associated to an overexpression (HDAC7 and 10) or an underexpression (HDAC6, SIRT2 and SIRT3) of these isoenzymes.

0725

THE CDK INHIBITOR CR8 DIMINISHES NFβB ACTIVITY IN PRIMARY CLL CELLS

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Background. Chronic lymphocytic leukaemia (CLL), often diagnosed in the elderly, is incurable with current therapeutic regimes. Recent studies have demonstrated that the microenvironment within lymphoid organs has a pro-survival effect on CLL cells, resulting in chemotherapy resistance. Novel therapies capable of overcoming such cytoprotective signals are therefore crucial. CLL cells exhibit constitutively active NFκB signalling, particularly in the lymph nodes (LN) microenvironment. Furthermore, the activation of Rel A (an NFκB transcription factor family member) acts as an independent poor prognostic marker for CLL. Therefore, inhibitors that target this pathway will greatly assist in the eradication of chemoresistant CLL cells. Recent findings indicate that cyclin-dependent kinase (CDK) inhibitors, which downregulate the levels of the anti-apoptotic Mcl-1 (a protein that plays a significant role in promoting the chemoresistance of CLL cells), can also inhibit NFκB signalling in T cells and myeloid leukaemia cell lines. **Aims.** This study was conducted to determine the mechanisms and the levels of inhibition of NFκB signalling by the Roscovitine analogue CR8 in primary CLL cells**. Methods.** Primary CLL cells were cultured in an *in vitro* co-culture system that closely replicates the LN microenvironment, and activates NFκB-mediated signalling pathways. Gene expression analysis was conducted by quantitative RT-PCR. Protein analysis was performed by western blotting. Nuclear Rel A activity levels in CLL cells were measured utilising an ELISA-based technique. **Results.** Our data establish that CR8 reduces transcriptional levels of NFκB family members (*REL*, *RELA*, *RELB*, *NFκB1* and *NFκB2)*, as a result of an inhibition of CDK9-dependent RNA polymerase II activity. CR8 treatment of CLL cells also stabilised the expression of IκBα, the cytoplasmic NFκB repressor, resulting in an inhibition of nuclear Rel A activity. These events resulted in the downregulation of the NFκB target gene cFLIP in CLL cells. **Conclusions.** Collectively, our study establishes that CDK inhibitors have the capacity to target key pro-survival microenvironmental signals that are generated in patient LNs both at a transcriptional level and by inhibiting NFκB DNA binding activity. This highlights the central role that these inhibitors could play as novel therapies to overcome CLL chemoresistance.

0726

AMONG VEGF RECEPTORS, THE NEUROPILIN-1 (NRP-1) REPRESENTS PROMISING, NOVEL TARGET FOR CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background. Angiogenesis might substantially contribute in the progression of chronic lymphocytic leukemia (CLL).VEGF bounds 3 types of ligands VEGFR1(Flt-1), VEGFR2(Flk-1/KDR), VEGFR3(Flt-4), exerting tyrosine kinase activity and two non-enzymatic receptors (neuropilin-1 and -2). Neuropilin - 1 (NRP-1) represents a receptor for VEGF, which has been reported to be overexpressed in several malignancies. NRP-1 expression was also found on plasmacytoid dendritic cells (PDC) as well as on regulatory T cells (Tregs). Both, PDC and Tregs represent cells that are involved in tolerance mechanisms. Thus, in addition to its role in angiogenesis, NRP-1 might be involved in tumor escape mechanisms, and expression of NRP-1 on tumor cells, Tregs and PDC might point to a promising target for therapy. **Aims.** In our study, we characterized mRNA levels of VEGF receptors including NRP-1 in a large cohort of CLL patients. Next the detailed characterization of NRP-1 on the surface level was performed. In functional studies the regulation of NRP-1 by VEGF was assessed. **Methods.** qRT-PCR was performed for the Flt-1, KDR, NRP-1 and FOXP3 (forkhead box P3) in 114 CLL patients. For characterization of the sur-

face expression of NRP-1 on leukemic B-cells, PDCs and Tregs in a group of 38 CLL patients and 7 healthy volunteers (HVs) by five parameter flow cytometric analysis was used. In functional studies, magnetically separated leukemic cells from 8 CLL patients were cultured with different VEGF concentrations and analyzed with flow cytometric afterwards. **Results.** The expression of NRP-1 on leukemic lymphocytes was significantly higher compared to NRP-1 expression on lymphocytes derived from HVs, with median of 22.72% vs 0.2%, p=0.0003. The median expression of NRP-1 on Tregs was 42.6 %. NRP-1 was expressed on almost all PDC with median expression 100%. While VEGF levels of 0.1 - 0.5ng/ml, which are in the range of VEGF concentration observed in CLL patient samples, effectively induced expression of NRP-1, higher VEGF concentrations inhibited NRP-1 expression. In a greater cohort of 114 patients, we observed correlation between Flt-1, NRP-1 and FOXP3 expression (r^2=0.53, p<0.0001 and r^2=0.49, p<0.0001, respectively). In the group with unmutated *IGHV* status the median expression of NRP-1 was 0.074 (p=0.02), in the group with mutated *IGHV* status we observed higher expression of NRP-1 (median 0.081). In patients without KDR expression we found higher level of FOXP3 compared to patients with expression of KDR (p=0.008). **Conclusions.** In conclusion, we found NRP-1 expression on CLL cells, and for the first time demonstrated that CLL derived Tregs as well as PDC also express NRP-1. Furthermore, our data provide evidence that NRP-1 expression might be regulated by VEGF levels in CLL. Thus, NRP-1 might represent an interesting link between angiogenesis and tolerance mechanisms. This functional link was confirmed by the correlations between expression levels of certain VEGF receptors and FOXP3 in a greater cohort of CLL patients. Therefore NRP-1 might represent an interesting molecule for therapy of CLL since it could target both leukemic cells as well as Treg and PDC cells responsible for escape of tumor cells from the immunosurveillance.

0727

CD200 EXPRESSION BY FLOW CYTOMETRY ACCURATELY DISCRIMINATES BETWEEN MANTLE CELL LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKAEMIA

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Chronic Lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) are CD5+ chronic lymphoproliferative disorders (CLPDs) that share many features. A differential diagnosis between CLL and MCL is vital due to their divergent clinical courses. The differential diagnosis is aided by immunophenotyping using markers such as CD23, FMC-7 and CD79b. A transmembrane protein, CD200, belonging to the immunoglobulin superfamily is expressed on resting and activated T and B cells, but not on NK cells, monocytes, granulocytes or platelets. It has been suggested to play a role in the differential diagnosis of these two diseases, although its expression in cases of monoclonal B cell lymphocytosis (MBL) and small lymphocytic lymphoma (SLL) has yet to be ascertained.The aim of this present study was to assess both the gene and protein expression of CD200 in a series of CLPDs. **Methods.** We assessed the expression of CD200 by RQ-PCR and flow cytometry. CD19+ lymphocytes were purified from peripheral blood (PB) of CLL patients (Miltenyi-autoMACS). Gene expression of CD200 was assessed using RQ-PCR. The expression of CD200 was assessed using MRC OX-104 (BD) on neoplastic cells from CLPD cases using flow cytometry. **Results.** There were 116 patients included in the study which consisted of: CD5 negative CLPD (HCL, MZL, FL), (n=22), MCL (n=23), CLL (n=58), MBL (n=10) and SLL (n=3).Relative quantification of CD200 mRNA expression was also carried out by RQ-PCR. Gene expression, reported as a fold change from CD19 positive normal donors showed no significant differences between CLL and MCL cases. CD200 was expressed on all CLL patients (CD200 mean=89%), MBL (63%), SLL (95%) and in all 23 MCL patients (3%) CD200 was negative. Twenty-two patients had CD5 negative CLPD (CD5- CLPD) with variable expression of CD200, with positivity observed in cases of HCL and MZL. The positive predictive value of CD200 for CLL was 97.5% with a sensitivity of 100% and a Pearson's correlation coefficient of 1. **Conclusions.** Our results confirm CD200 expression in the neoplastic cells of CLL patients but not in MCL. This expression is controlled at the translational level as no significant differences existed when CD200 gene expression was compared between MCL and CLL cases normalized to CD19 positive controls.Our results also demonstrate that the antigen was expressed in the neoplastic cells of MBL and SLL patients. Our findings also confirm previous reports of CD200 positivity in CD5- CLPD such as HCL and MZL.We suggest that CD200 should be included in routine flow panels to differentiate between MCL and CLL cases

0728

TELOMERES SHORTEN DURING CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) EVOLUTION: AN ANALYSIS ON 88 CASES

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Background. Telomere length (TL) at diagnosis has been established as an independent outcome predictor in CLL (Rossi, Leukemia 2009). However data on TL dynamics over time are scant and anedoctal. **Aims.** Aim of this study was to evaluate telomere dynamics in the natural history of CLL. This issue has been here addressed on a series of 88 CLL patients (pts).**Methods:** 25 pts were assessed for TL at diagnosis and at relapse and 63 pts had two determinations during the 'watch and wait' (WW) phase. The series was characterized in terms of Binet stage, ALC, CD38, ZAP-70, IGHV mutational status (IGHV-MS), stereotyped receptors, cytogenetics and clinical history. LDH, B2-microglobulin, p53 mutations and CD49d were available in more than 70% of pts. TL was analyzed as previously described (Rossi, Leukemia 2009; Ladetto, Blood 2004). Median time between TL determinations was 44 months (range 12-231). Telomere loss was calculated in terms of absolute loss (AL) and yearly loss (YL). Continuous variables were compared by the Mann-Whitney test, while TFS by the stratified Kaplan-Meyer method. **Results.** Telomeres were shorter at follow-up compared to baseline (median loss of 651bp, range +493bp, -5874bp; p<0.001) (Fig 1A). AL and YL were greater in cases with higher baseline TL, while those with short telomeres at diagnosis had only modest additional erosion (p=ns for pts in the 25th lowest percentile) (Figure 1B). Telomere loss over time was noticeable both in pts assessed at diagnosis and at relapse as well as in those assessed during the WW phase, but clearly inferior in the former subgroup (YL of -61bp, p<0.05 and -210bp, p<0.01), possibly due to the higher number of patients with short telomeres. AL and YL did not correlate with any available clinical or biological parameter, with the exception of a positive association with IGHV-MS (p<0.05). Considering the sole WW population, with a clinical follow up of 78 months and a median TFS of 43 months, pts with baseline TL shorter than the validated cut-off value of 5000bp (Rossi, Leukemia 2009) had an inferior TFS (median TFS 41 vs 182 months; p<0.0001) as expected. Binet status and IGVH-MS were also predictive for TFS in this series. Surprisingly, also an YL above the median value (-210bp) appeared to be predictive for an inferior TFS (median TFS 82 vs 182 months; p<0.05) (Figure 1C). Following stratification of pts according to baseline TL (< or > 5000bp), YL was predictive for TFS in patients with long and stable telomeres (Figure 1D). **Conclusions.** The results of the first systematic analysis on TL dynamics in CLL indicate the following: i) progressive telomere erosion occurs as part of the natural history of CLL; ii) telomere loss is more pronounced when baseline TL is higher; iii) accelerated telomeric loss associates to an inferior TFS. The results described in the present analysis corroborate basic studies suggesting that telomere disruption represents a critical step associated to CLL progression.

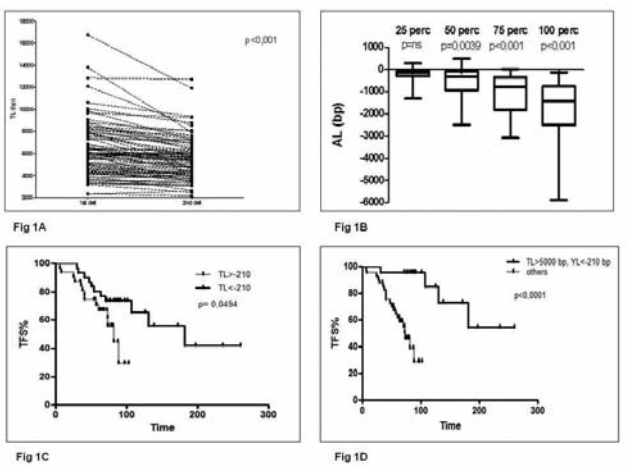

Figure 1.

0729

CD80 AS A TARGET OF MIR-146 IN CHRONIC LYMPHOCYTIC LEUKEMIA ASSOCIATED WITH AUTOIMMUNE HEMOLYTIC ANEMIA

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Background. CD80 is a protein found on activated B cells and monocytes that provides a costimulatory signal necessary for T cell activation and survival. CD80 is the ligand for two different proteins on the T cell surface: CD28 (for autoregulation and intercellular association) and CTLA-4 (for attenuation of regulation and cellular disassociation). MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate mRNA translation and play an important regulatory role in most biological processes. We have previously shown that chronic lymphocytic leukemia (CLL) associated with autoimmune hemolytic anemia (AIHA) presents a distinctive miRNA signature, including a downregulation of miR-146b, which has been related to CLL and autoimmunity. **Aims.** The main objective of this study was to determine targets of miR-146 that can play a role in the the development of AIHA in CLL patients. **Methods.** Using Tarbase, a bioinformatic tool, we identified the putative targets of miR-146, including CD80. Two cell lines (HDMYZ and Ramos) were transfected with 1µg of modified psiCHECK-2 vector, which contains a fragment where miR-146 binds of the 3'UTR region of CD80, plus 200nM of pre-miR-146 or a pre-miR-negative control. Renila/luciferase luminescence was evaluated with the Dual Luciferase Promega kit. In order to examine the effect of miR-146 on protein levels, cells were transfected with 200nM pre-miR-146 or negative control, and CD80 levels on cell surface were evaluated by flow cytometry. CD80 was also evaluated on cryopreserved cells from five patients with CLL associated with AIHA and twelve with CLL not associated with autoimmunity. **Results.** Renila/luciferase levels were lower in both the HDMYZ and Ramos cell lines after transfection with pre-miR-146 than in cell lines transfected with the negative control (72.3 % and 80.4%, respectively) (Figure 1A). Although HDMYZ cells normally express low levels of CD80, we observed a 3.7% reduction of positive cells in HDMYZ cells transfected with pre-miR-146 compared to those transfected with the negative control (14.0% vs. 17.7%, respectively; p=0.015). After transfection with pre-miR-146, Ramos cells also showed a reduction of CD80+ cells (28.7% vs. 41.3%; p=0.020) (Figure 1B). Finally, patients with CLL associated with AIHA had higher levels of CD80 than those without autoimmunity (13.4% *vs.* 9.3% respectively; p=0.027). **Conclusions.** CD80 is a target of miR-146, which may account for the increased levels of CD80 in CLL associated with AIHA. Further analyses are warranted to ascertain the role of CD80 and miR-146 in B-/T-cell synapse in CLL associated with AIHA.

DIAGNOSIS (N=161)*	N (%)	CD200 (median, range)	CD200≥20%, N (%)
CLL/SLL	61 (37.9)	100 (23-100)	61 (100)
CLL-like MBL	22 (13.7)	100 (70-100)	22 (100)
Atypical CLL MBL	6 (3.7)	94 (50-99)	6 (100)
CD5-negative MBL	2 (1.2)	51 (33-70)	2 (100)
MCL	16 (10)	1 (0-83)	4 (25)
SMZL	28 (17.4)	41 (0-100)	18 (64)
Splenic B-cell lymphoma/leukemia, unclassificable	1 (0.6)	73	1 (100)
HCL	7 (4.4)	100 (30-100)	7 (100)
FL	5 (3.1)	32 (0-86)	3 (60)

Figure 1. Validation of CD80 as target of miR-146.

0730

CD200 EXPRESSION DETERMINED BY FLOW CYTOMETRY IN MATURE B-CELL NEOPLASMS WITH PERIPHERAL BLOOD INVOLVEMENT

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Background. CD200 is an immunoglobulin superfamily membrane glycoprotein widely expressed in multiple cell types. Its expression has been reported in non-hematological malignancies and also in some hematological diseases. Recent studies have demonstrated that CD200 expression may be helpful in the differential diagnosis of chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). **Aims.** To analyze the expression of CD200 by flow cytometry (FC) on neoplastic cells in a series of patients consecutively diagnosed with mature B-cell neoplasms with peripheral blood (PB) involvement. **Methods.** PB samples of 150 patients diagnosed from October 2009 until June 2011 were evaluated by four-color FC with a panel of monoclonal antibodies (moAb) currently used in our center to study mature B-cell neoplasms. This panel included CD200-PE (BD Pharmingen, clone MRC OX-104) in combination with CD19-PerCP-Cy5.5 and CD5-APC. At least 30,000 events were acquired in a FACSCanto II flow cytometer (BD) and analyzed with the FACSDiva software. The expression of CD200 was assessed with respect to the mean fluorescence intensity observed in cells labeled with a matched isotypic moAb. The diagnoses were established according to the WHO 2008 classification (Table 1). In nine cases a definitive diagnosis was impossible to be established (SMZL-v vs HCL-variant, 1 case; CLL vs LPL, 2; CLL vs SMZL CD5+, 4; mature B-cell neoplasm different to CLL, 2).

Table 1.

DIAGNOSIS (N=161)*	N (%)	CD200 (median, range)	CD200≥20%, N (%)
CLL/SLL	61 (37.9)	100 (23-100)	61 (100)
CLL-like MBL	22 (13.7)	100 (70-100)	22 (100)
Atypical CLL MBL	6 (3.7)	94 (50-99)	6 (100)
CD5-negative MBL	2 (1.2)	51 (33-70)	2 (100)
MCL	16 (10)	1 (0-83)	4 (25)
SMZL	28 (17.4)	41 (0-100)	18 (64)
Splenic B-cell lymphoma/leukemia, unclassificable	1 (0.6)	73	1 (100)
HCL	7 (4.4)	100 (30-100)	7 (100)
FL	5 (3.1)	32 (0-86)	3 (60)
LPL	2 (1.2)	60 (40-81)	2 (100)
MALT lymphoma	2 (1.2)	44 (40-48)	2 (100)
Without definitive diagnosis	9 (5.6)	74 (0-100)	7 (78)

*10/150 patients (6.7%) with two (N=9) or more (N=1) B-cell populations.
CLL, chronic lymphocytic leukemia; SLL, small lymphocytic lymphoma; MBL, monoclonal B-cell lymphocytosis; MCL, mantle cell lymphoma; SMZL, splenic B-cell marginal zone lymphoma; HCL, hairy cell leukemia; FL, follicular lymphoma; LPL, lymphoplasmacytic lymphoma; MALT, mucosa-associated lymphoid tissue.

Results. In ten out of the 150 patients (6.7%) two (N=9) or three (N=1) B-cell populations with an abnormal phenotype were identified. The percentage of neoplastic cells with CD200 expression observed in the different diseases and the number of cases with CD200 expression ≥20% is shown in the table. An expression of CD200≥90% was observed in 59/61 (97%) CLL/SLL cases, 20/22 (91%) CLL-like MBL, 5/6 (83%) atypical CLL MBL, 0/2 (0%) CD5-negative MBL, 6/7 HCL (86%) and 0/16 (0%) MCL. In the remaining pathologies CD200 expression was highly heterogeneous. CD200 expression was similar in CLL and CLL-like MBL but significantly different between these and MCL (CD200≥ vs <20%, *P*=0.011). In addition, the expression of CD200 in MCL patients with conventional clinical behavior (N=7) was significantly lower than that observed in cases displaying an indolent clinical course (N=9, median survival without

treatment, 82 months; range, 15-167) (CD200≥ vs <5%, *P*=0.011). In the majority of HCL patients the expression of CD200 was ≥90%. CD200 expression in SMZL was variable and in some cases (7/28, 25%) similar to that observed in CLL. **Conclusions.** In our series, an expression of CD200≥90% was observed in nearly all cases of CLL, CLL-like MBL and HCL, being this expression significantly different from that observed in MCL. The expression of CD200 in MCL patients with an aggressive clinical course was significantly lower than that observed in MCL cases with an indolent behavior, data that need to be confirmed in longer series. Patients diagnosed with SMZL displayed a highly variable CD200 expression, in agreement with the clinical and biological heterogeneity of this entity.On behalf of Grupo Español de Citología Hematológica (GECH).This study has been funded in part by FIS PI10/00366.

0731

PROLIFERATIVE AND APOPTOTIC STATUS ON LYMPH NODE SECTIONS, INCLUDING THE PROLIFERATION CENTERS (PC), OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS

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Background. Recent data suggest that PC play an important role in the biology of CLL, as they constitute its proliferative compartment. However, the complexity of the microenvironment as well as the molecular events that take place in PC and their clinical significance are not completely elucidated. **Aims.** To identify the presence of PC in lymph node and other tissues; to analyze their proliferative status; to evaluate the expression of molecules implicated in the apoptotic process in PC, compared to their expression in the nonPC areas; and to correlate the aforementioned findings with the clinical and laboratory features in a series of CLL patients. **Patients and Methods.**Fifty patients, fulfilling the diagnostic criteria of CLL/SLL were enrolled in this study. All necessary clinical and biological data were recorded on each patient. In all tissue sections(44 lymph nodes,5 spleens, 1skin), in the PC and the nonPC areas, the following molecules participating in the proliferative/apoptotic process were studied and evaluated by immunohistochemistry: Ki-67, Bcl-2, Fas, FasL, c-FLIP, survivin and cleaved caspase3 that was evaluated in the entire tumor area.RESULTS-Median patients' age was 55 years (36-77). Twenty-nine (58%) had disease stage A, 16(32%) B and 5(10%) C.PCs were easily identified after staining with H+E both in lymph node and splenic sections. In 17 cases the major diameter of PCs exceeded the diameter of a x20 power field and therefore were considered to be expanded. Median Ki67 proliferation index per proliferation center was 10% (1-20%), while median Ki67 in the whole tissue section was 3% (1-8%) (p<0,0001).Median Fas, Fasl and c-FLIP expression in the PCs was 50%, 30% and 50% respectively, whereas the respective values in the nonPC areas were 40%, 10% and 40%.Only the difference noted in the FaSL expression between PCs and nonPC areas was statistically significant(p= 0.0107) Moreover, FasL expression in the PCs was positively correlated with Fas and c-Flip expression in the same areas (p=0.018 for Fas and FasL, p=0.0154 for FasL and c-Flip). Survivin expression was detected in all cases with the percentage of positive neoplastic cells ranging from 1% to 80%. In half of the cases survivin expression in the PCs was higher than that of the rest tumour compartment. Those cases displayed also increased overall survivin expression (p=0.0266) and higher c-FLIP expression in the PC areas when compared to the nonPC areas (p=0.017).Strong homogenous Bcl-2 expression was observed both in PC and in the nonPC areas.In multivariate survival analysis concurrent overexpression of Fas/Fasl and c-FLIP in the PCs emerged to be an independent predictor of worse outcome for OS (p=0.042) as well as for DFS (p=0.008)CONCLUSIONSPC in lymph nodes and spleen of CLL pts present particular proliferative and apoptotic features.The correlation of Fas, FasL and cFlip in PCs implicates cFlip as an inhibitor of Fas induced death pathway in CLL patientsConcurrent overexpression of Fas,FasL and cFlip in PCs was correlated with patients' survival.

0732

THE ROLE OF CD200 IN THE DIFFERENTIAL DIAGNOSIS OF MATURE B-CELL NEOPLASMS BY FLOW CYTOMETRY

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Background. Multiparameter flow cytometry is a useful tool to the diagnostic evaluation of mature B-cell neoplasms (MBN). The combination of CD5 and CD10 restricts the diagnostic possibilities, assisting in the selection of subsequent. **Methods.** The most frequent association is CD5+/CD10-, whose main hypotheses are: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL) and atypical CLL. In this subgroup, CD23+ cases are usually classified as CLL; and CD23- cases, as MCL. However, the immunophenotype of CLL and MCL cases frequently overlaps, and the pattern of CD23 expression is not helpful for the diagnosis of most cases of atypical CLL . In addition, other MBN may have atypical expression of CD5, further complicating the differential diagnosis in this subgroup. Recently, it has been shown that CD200 may improve the distinction between CLL (CD200+) and MCL (CD200-), but the role of CD200 expression in atypical CLL and other MBN remains to be established. **Aims.** Investigate the expression of CD200 in 159 consecutive cases of MBN diagnosed over a period of 11 months. **Methods**: Expression of CD200 (clone OX104) was evaluated on PB, BM and lymph node samples in a FACSCalibur flow cytometer using a large panel of MAbs. Cases were classified as CD5+ MBN: 81 cases [typical LLC, 45; atypical CLL, 11; MCL, 14; CD5+ B-cell lymphoma, 11]; CD10+ MBN: 16 cases [follicular lymphoma (FL), 11; diffuse large cell lymphoma (DLCL), 5]; CD5-/CD10- MBN: 31 cases; lymphomas with hairy/villous lymphocytes, 20 [hairy cell (HCL), 13; splenic marginal zone lymphoma (SMZL), 6; unclassifiable, 1]; prolymphocytic leukemia (PL), 7; and lymphoplasmacytic lymphoma (LPL), 4. **Results.** In the CD5+ group, CD200 was expressed with high intensity in all cases of typical CLL (IMF= 284.1), but was absent in all MCL cases (p <0.0001). All cases of atypical CLL also expressed CD200, but with lower intensity than typical CLL (IMF = 153.3, p = 0.002). Among the CD5+ B-cell lymphomas, 9/11 cases expressed CD200 (IMF = 112.7). In the CD10+ group, CD200 was expressed with dim intensity in 8/11 FL (IMF = 39.8), and in 5/5 cases of DLCL (IMF = 40). All cases of HCL expressed CD200 with high intensity (IMF = 464.4), while SMZL cases had a lower intensity (IMF = 68.6, p <0.0001), with one negative case. The expression of CD200 was heterogeneous in CD5-/CD10- lymphomas, and expressed in 21/31 cases (IMF = 78.6). In PL, 2/7 patients were positive (IMF = 98.7); and in LPL, 2/4 cases (IMF = 46.6). **Summary and Conclusions.** CD200 is strongly expressed in CLL and is an excellent marker for the differential diagnosis with MCL, even in cases with atypical phenotype. Furthermore, CD200 is highly expressed in HCL, being useful in the differential diagnosis with SMZL. However, lack of CD200 is not an exclusive finding of MCL, being also observed in other MBNs, including PL and CD5+ B lymphomas. These results expand the understanding of the CD200 expression in MBNs, giving further support for the inclusion of this marker in the flow cytometric panels for the differential diagnosis of MBNs.

0733

CD200 IN DIAGNOSIS OF CHRONIC LYMPHOPROLIFERATIVE DISORDERS- A STUDY OF 122 PATIENTS

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Background. The diagnosis and management of patients with chronic limphoproliferative diseases has become dependent on immunological criteria. Flow cytometry immunophenotyping is used for rapid and specific diagnoses. But, there are cases when we are not facing a typical immunophenotype and for that, there is a constant need for finding new markers and new combinations of markers that allow us to improve and develop our diagnoses. Mature B-cell lymphoproliferative disorders have specific phenotype and in the presence presence of a CD5 positive B-cell lymphocytosis, it is important to differentiate Chronic Lymphocytic Leukemia (CLL) from mantle cell lymphoma (MCL). **Aims.** Our aim was to evaluate CD 200 expression in different B-cell chronic lymphoproliferative disorders. CD200 is a membrane glycoprotein belonging to the immunoglobulin superfamily and overexpression of CD200 has been reported in a number of malignancies, including CLL, as well as on cancer stem cells. **Methods.** We analyzed CD200 expression in 122 patients diagnosed with chronic lymphoproliferative disorders(100 patients with CLL, 10 patients with splenic marginal zone lymphoma (SMZL), 10 patients with MCL and 2 patients with hairy cell leukemia), in the Hematology Department of Emergency University Hospital Bucharest.We performed immunophenotypical analysis of peripheral blood and bone marrow aspirate on BD FACS Calibur flowcytometer .Our diagnose panel included the following markers: CD19, CD20, CD5, CD23 , CD79B , CD103, CD11c, CD25, CD10, FMC7. **Results.** CD200 was brightly expressed in all 100 CLL patients (100%). In SMZL patients, CD200 was dim positive (40%-60%), in patients with HCL. CD200 was also bright positive (96% and 97%) and in patients with MCL CD200 was negative (1-10%); CD 200 was significantly higher in CLL patients compared with other B-cell chronic lymphoproliferative disorders. We found 14 patients with CD19, CD5 positive population and CD23-, but with high expression of CD 200. Cyclin D1 was negative on bone marrow biopsy in 13/14 of these patients. (1/14patients was without bone marrow involvement). **Conclusions.** CD200 has a great impact in diagnoses of B-chronic lymphoproliferative disorders, especially when we want to determine the origine of a CD19, CD5 positive population and differentiate CLL from MCL. CD 23 is a reliable marker in those cases, but, as we showed, CD23 might have a lower specificity than CD200 for CLL. The diagnosis of MCL has to be confirmed by detection of cyclin D1 positivity or by the presence of the t(11;14)(q13;q32) chromosomal translocation detected by cytogenetics, Western blot, Polymerase Chain Reaction (PCR) analysis, fluorescence in situ hybridization (FISH). But, these methods are expensive, time-consuming and not quite available.We added CD200 in our panels for diagnoses of chronic lymphoproliferative disorders, not to replace CD23, but to improve our diagnoses.

0734

EXPRESSION OF BCL2L12 GENE IN CHRONIC LYMPHOCYTIC LEUKEMIA: ASSOCIATION WITH CLINICAL AND MOLECULAR PROGNOSTIC MARKERS

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Background. Chronic lymphocytic leukemia (CLL) manifests as clonal expansion of mature $CD5^+$ $CD19^+$ $CD23^+$ $sIgM^{low}$ B lymphocytes, whose gradual accumulation in blood, bone marrow and lymphoid organs is attributed primarily to impaired apoptosis. Resistance to apoptosis results from microenvironmental survival signals, as well as from inherent dysregulation of apoptotic machinery in malignant B lymphocytes. Genetic alterations and aberrant expression of Bcl2 family proteins have been described in CLL, such as overexpression of Bcl2, $BclX_L$ and Mcl1, and increased Bcl2/Bax ratio. Bcl2L12 is a novel member of Bcl2 family of apoptosis regulators, whose pro- or anti-apoptotic role has not been fully elucidated yet. Nevertheless, its altered expression relative to normal tissue has been reported in several types of cancers. **Aims.** The aim of this study was to analyze the expression of Bcl2L12 in CLL, and to assess the association of Bcl2L12 mRNA levels with clinical features of patients. Furthermore, the association with molecular markers which exert the greatest prognostic value was studied, namely the mutational status of rearranged immunoglobulin heavy variable (IGHV) genes, CD38 and lipoprotein lipase (LPL) gene expression. **Methods.** This study enrolled 58 unselected CLL patients and 10 healthy controls. The expression of Bcl2L12 gene was analyzed in peripheral blood mononuclear cells by RQ-PCR methodology, using SYBR Green chemistry. LPL expression levels were also measured by RQ-PCR, using TaqMan chemistry. In all RQ-PCR experiments, Abl was used as endogenous control gene. Quantification of target gene expression was made by comparative ddCt method, using HL-60 cell line as the calibrator. CD38 status was determined by flow cytometry, and IGHV mutational status by sequencing of clonal IGHV rearrangements and sequence analysis by ImMunoGeneTics database and tools. **Results.** RQ-PCR expression analysis of Bcl2L12 revealed significantly higher levels of Bcl2L12 mRNA in CLL samples in comparison to normal blood samples (p=<0.001). ROC curve analysis showed that Bcl2L12 expression efficiently discriminates CLL cases from healthy controls (ROC curve area = 0.8733; 95% confidence interval = 0.7781-0.9685; p=0.0002) . Bcl2L12 mRNA levels showed no association with either gender, age at diagnosis, Binet stage or outcome of the disease (progressive vs. non-progressive). Although Bcl2L12 expression was negatively correlated to lymphocyte doubling time (LDT) and positively to β2-microglobulin levels, in both cases this correlation did not reach statistical significance. On the other hand, Bcl2L12 levels were significantly elevated in patients with abnormal vs. normal LDH values (p=0.038). Regarding molecular prognostic markers, no association was found between Bcl2L12 mRNA levels and either IGHV mutational status, CD38 status or LPL expression. **Conclusions.** Our results demonstrated a significant overexpression of Bcl2L12 gene in CLL patiens in comparison to healthy controls, implying its role in the pathogenesis of the disease. However, relatively homogenous Bcl2L12 mRNA levels among patients did not reflect their clinical characteristics (with the exception of LDH status), and failed to show association with molecular markers predictive of prognosis in CLL.

Chronic lymphocytic leukemia - Clinical 2

0735

AN INNOVATIVE BIOMEDICAL SCIENTIST LED REMOTE MONITORING PROGRAMME FOR STABLE EARLY CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. Early Chronic Lymphocytic Leukaemia (CLL) is, with increasing frequency, diagnosed after 'routine' blood tests reveal lymphocytosis, predominantly in the elderly. CLL is frequently indolent, requiring no treatment. Patients can be frail with co-morbidities. We practise in a rural area where patients travel up to 25 miles to clinics. Increasing demand for our services are making clinics more congested. **Aims.** We have, with dual aims of avoiding unnecessary travel for patients and over-booking of clinics, piloted a biomedical scientist (BMS) led remote monitoring service for patients with stable early CLL. The extended role of the BMS fits in well with the modernising scientific careers pathway recently implemented in the UK. We report here results of our first 3 years of the programme. **Methods**. Patients with stage A CLL were identified from haematology clinic registers. Entry criteria included confirmed CLL stage A by clinical examination, morphology and immunophenotyping with CLL score of at least 4/5, minimum 1 year follow up from diagnosis, lymphocyte doubling time of >12 months and written informed consent. CD38 status was available in some cases, but CD38+ patients were not excluded. Information sheets were provided to patient and GP. Six monthly FBC and 12 monthly DCT, LFT and immunoglobulins were requested by posting blood forms for patients to have samples taken locally. The BMS conducted a 6 monthly telephone interview with the patient using a proforma which enquired about frequency of infection, weight loss, sweats or obvious lymphadenopathy as well as whether there was any issue they wished to discuss with their Haematologist.Results of questionnaires and corresponding blood tests were reviewed by the Consultant Haematologists. Patients with satisfactory results were re-tested as per protocol. Patient and clinician could request out-patient clinic review if deemed necessary by either party. A longer testing interval (12 months) was introduced in selected patients with long duration of stable disease.Hard copies of completed questionnaires were filed in the department. Blood test results were available electronically. After each interaction patients received a letter, copied to their GP, advising the outcome. A patient satisfaction survey of the service was recently undertaken. **Results**. Sixty three patients have been enrolled to date; 4 have died (none from CLL), 7 have been recalled for clinic review; 5/7 were subsequently returned to the monitoring programme. Two patients have requested annual clinic review with remote monitoring in-between. We estimate that at least 140 out-patient appointments per year have been saved. Forty patients responded to our satisfaction survey; all were happy with the programme. **Conclusions.** We have shown that a BMS led remote monitoring programme for early CLL is feasible and safe, with benefits for patient and clinician and a high level of patient satisfaction. We now plan to extend the programme to other areas of our Health Board, and with the implementation of the All-Wales Laboratory Information System (LIMS) in late 2012, there may be scope to extend further.

0736

NEW PROGNOSTIC SCORE BASED ON COMORBID CONDITIONS AND BETA-2 MICROGLOBULIN FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. The clinical outcome of patients with chronic lymphocytic leukemia (CLL) is variable. The ability to predict the clinical course in patients with early stage CLL has been markedly improved by the use of molecular prognostic markers. Most of these data come from patients participating in clinical trials. Such trials typically have strict eligibility criteria based on performance status and organ function. Little is known about the organ function of unselected patients (i.e., regardless of whether they are eligible for trials). Adult comorbidity evaluation-27 (ACE-27), Cumulative Illness Rating Scale (CIRS) and hematopoietic cell transplantation comorbidity index (HCT-CI) are comorbidity indexes that take concurrent presence of nonmalignant diseases into account when explaining survival. They differ in both the number and categorization of comorbidities. **Aims.** We investigated the prognostic significance of three comorbidity indexes in adition to standard prognostic tools in a large sample of patients with CLL.Methods: We retrospectively evaluated the impact of ACE-27, CIRS and HCT-CI in a cohort of 115 adult patients with chronic lymphocytic leukemia (HLL). Also we tested impact od standard prognostic tool available in daily practice as age, gender, Rai/Binet clinical stage, LDH level, type of bone marrow infiltration and CD38 expression. Patient and survival data were acquired from inpatient hospital records. Cox hazard regression model was used to analyze overall survival according to standard prognostic factors and comorbidities classified by the ACE-27, CIRS and HCT-CI. **Results.** Median follow up of our group was was 39 months while median overall and progression free survival was 80 and 37 months, respectively . We found a significant association between ACE-27 and beta-2 microglobulin (b2M) and overall survival (p=0,011; RR=2,215; 95%CI for RR 1,203-4,076 and p= 0,014; RR= 2,971; 95%CI for RR 1,247 - 7,075, respectively). Other two comorbidity indexes - CIRS and HCT-CI and standard prognostic factors did not outperform the survival model, while in multivariate analysis ACE-27 remained the most important predictor of overall survival (p=0,009). Median overall survival time between ACE groups (none/mild *vs.* moderate/severe) was 94 *vs.* 56 months, respectively (Log rank=6,973; p=0,008). Also, patients with b2M < 3mg/ml had significantly longer overall survival (Log rank=6,721; p=0,010). We developed a new scoring system based on the ACE-27 score value and serum levels b2M. Patients with a score of 0 (low values of ACE and b2M) did not reach median overall survival while patients with a score of 2 (increased levels of ACE-27and b2M) had a significantly shorter overall survival of 34 months (p<0,0001). **Conclusions.** In our group of adult CLL patients the overall survival is associated with the presence of comorbidities defined by the ACE-27 index. ACE-27 and b2M present a superior comorbidity risk-adjustment model for HLL survival prediction.

0737

CYTOXAN AS FIRST-LINE THERAPY IN LGL LEUKEMIA : REPORT OF A SERIES OF 21 PATIENTS

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Background. Treatment of large granular lymphocytes (LGL) leukemia is based on immunosuppressive agents like methotrexate, ciclosporine or cytoxan. Given the lack of prospective studies, no standard regimen has been defined. Empirically, low dose oral methotrexate (MTX) is mainly used as first line therapy, except in LGL leukemia associated with pure red cell aplasia in which cytoxan has shown encouraging response rate. Among the few available retrospectival studies, continuous oral administration of low dose cytoxan appears to be efficient in LGL leukemia regardless of the treatment indication (neutropenia or anemia). Moreover, it has been reported that patients can respond to cytoxan after MTX therapy failure. **Aims.** We analysed retrospectively the results of cytoxan used as first-line therapy in LGL leukemia.**Methods:** Diagnosis criteria of LGL leukemia were those previously published (Lamy T, Blood 2011). Cytoxan was delivered at 100 mg/day except for one patient (200 mg/week), during 4 to 6 months. Five patients received concomitantly steroid during the first weeks. Response was evaluated after month 4. Complete response (CR) was defined as the complete normalization of blood counts and circulating LGL lower than $0.3x10^9$/L. Partial response (PR) was defined as an improvement in blood counts which do not meet criteria for CR (neutrophil increasing more than 50% and reaching more than 0.5 but less than $1.5x10^9$/L with non-recurrence of infections, or hemoglobin level increasing more than 2 g/dL from baseline and transfusion requirements stopping without normalization of the hemoglobin level. **Results.** Twenty one patients suffering from LGL leukemia (T cell n=18 and NK subtype n=3) were included in this study. There were 14 females and 7 males with a median age of 72 years. Diseases related to LGL leukemia were as follows: rheumatoid arthritis (n=2), chronic inflammatory bowel disease (n=1), dysthyroidism (n=3), pulmonary hypertension (n=2). Hemoglobin, neutrophil, and LGL median level counts were 12 g/dl, 0.5 G/L and 3.8 G/L respectively. Reasons for starting therapy were: isolated severe neutropenia (n=12) including two patients with severe infection, anemia (n=5), pulmonary hypertension and neutropenia (n=2), and two for other reasons (thrombocytopenia and auto-immune disease related to LGL leukemia). The overall response rate (ORR) was 71% (15/21) with 12 CR (57%) including 2 molecular CR,3 PR (14%), stable disease 4/21 (19%) and 2 failures/21 (10%). The ORR for neutropenic and anemic patients were 75% (9/12) and 60% (3/5), respectively. Median time to best response was 9 months. With a median follow-up of 27 months (4-98), only one out of the 15 responders has relapsed. PFS is 83% at 24 months. **Conclusions.** This largest series of cytoxan used as a first-line therapy in LGL leukemia shows encouraging results with an ORR of 71%. In comparison, ORR obtained with methotrexate and ciclosporine are

55% and 56%, respectively. Interestingly, cytoxan showed promising results in both neutropenic and anemic patients. Furthermore, compared to methotrexate, cytoxan treatment time is shorter, with longer response duration and less toxicity. These results support a prospective randomized study comparing cytoxan and methotrexate which is currently ongoing.

0738

SIGNIFICANCE OF BONE MARROW RETICULIN FIBROSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA: A STUDY OF THERAPY 'NAÏVE' PATIENTS WITH PROGNOSTIC IMPLICATIONS

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Background. Lymphocytes have been implicated in the development of marrow fibrosis. An increased reticulin network is seen in a variety of lymphoproliferative disorders, most prominently in hairy cell leukemia. Bone marrow (BM) biopsies from patients with chronic lymphocytic leukemia (CLL) may show different patterns of reticulin fibrosis but its incidence and prognostic significance have not been extensively studied and remain unclear. **Aims.** To assess the presence and possible significance of BM reticulin fibrosis in patients with treatment "naive "CLL and to attempt to correlate the degree of fibrosis with some of the well recognized clinical and laboratory prognostic parameters forCLL , as well as overall survival (OS) and eventual outcome in this patient cohort.Methods: We retrospectively reviewed data of untreated patients withCLL seen and followed at 9 medical centers inIsrael between1987-2012. BM trephine biopsies of all patients were evaluated for the presence and pattern of reticulin fibrosis before anti-leukemic therapy was started. Grading of fibrosis was established using a scoring system comprising four grades (0-3), based on the 2005 European consensus report. Prognostic and predictive factors forCLL outcome including: age, gender, Binet and Rai stages, CD38%, CD23%, Zap70 positivity determined by flow cytometry, serum beta 2 microglobulin levels, pattern of BM infiltration and spleen size were compared with grade of reticulin fibrosis, as well as OS and eventual outcome . **Results.** Our final cohort included 170 patients (150 males and 20 females). Median age at diagnosis was 63 years (range 32-86) and 5 years OS was 77.1%. The Binet staging system easily separated the cohort into 3 prognostic groups. Grading and the extent of reticulin fibrosis correlated with overall survival and mortality (p=0.003) readily dividing the patient cohort into four categories of survival curves according to the patterns defined (grade 0-3).Patients with reticulin fibrosis grade 2 had higher risk of mortality compared to grade 0 (H*azard Ratio* of *4.25)* and this trend was even more evident for patients with grade 3 fibrosis *(*H*azard Ratio* of *5.718)*.Advanced fibrosis (grade 3) was associated with thrombocytopenia (platelet<100.000mm^3)(p=0.025), anemia (hemoglobin<12.1 gr/L)(p=0.016), B2mg levels of > 4000 µg/mL (p=0.0031) , low expression of CD23 (p=0.0054) and CD38 positivity onCLL cells (p=0.03). There was no correlation between the extent of fibrosis and spleen size, pattern of BM infiltration (non diffuse versus diffuse), proportion of infiltrating CLL cells on bone marrow aspiration, level of lymphocyte count in the peripheral blood or Zap70 positivity in the leukemic cells . **Conclusions.** We identified a correlation between the grade of BM reticulin-fibrosis and poor survival in patients withCLL. This also correlated with the presence of very high levels of serum beta2 microglobulin and increased CD38 expression onCLL cells. Increased reticulin -fibrosis in the bone marrow appears to have significant prognostic implications for these patients. The simple histo-chemical staining procedure for detecting reticulin can easily be added routinely when examining BM biopsies of patients withCLL and can provide useful information relating to prognosis in these patients

0739

COMPARATIVE ANALYSIS OF JAPANESE AND EUROPEAN CLL PATIENTS

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Background. Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in Western countries but very rare in Eastern Asia. In studies among migrants this low incidence is maintained speaking in favor of a relevant hereditary predisposition. Such genetic factors could also account for significant differences in the biology of the disease. However, to date the impact of prognostic markers and drug therapy have been mainly studied among Caucasians. **Aims.** The aim of this retrospective study was to establish a framework for comparative studies between Japanese and European patient cohorts and to identify relevant factors, which need to be considered for prospective investigations. **Material and Methods.** CLL patient databases comprising clinical and molecular data from 316 Austrian patients diagnosed between 1973 and 2011 at the Medical University of Vienna and from 96 Japanese patients diagnosed between 1988 and 2007 at centers of the CLLRSG were analyzed. Patients with incomplete data were included in the analysis only for any data items that were available. An additional analysis, adjusted for Binet stage, was performed. **Results.** Several significant differences between the two patient populations were observed (Table 1). Japanese patients were older at diagnosis and the disease was more advanced. The latter was reflected in a significantly higher proportion of Binet B and C stages, more patients with elevated lactic dehydrogenase (above the institutional upper limit of normal) as well as lower mean platelet counts and hemoglobin concentration in the Asian cohort. The male to female ratio was 1.5:1 in both CLL populations. Interestingly, we found a notably higher proportion of atypical, surface CD23 negative, cases among Japanese CLL patients (33.8% of cases, compared to 2.3% in the European cohort, p<0.001). In contrast, the proportion of CD38 positive cases was almost twice as high among the Caucasians (30.6% vs. 17.6%, p=0.059). In unadjusted comparison, median treatment free (TFS) and overall survival (OS) were also shorter among Japanese patients, especially in the subset of typical CLL patients only.In Binet adjusted analysis among all patients, impact of ethnicity on TFS and OS lost significance. Differences in other markers remained, albeit less pronounced in most cases. In analysis of typical CLL patients only, Japanese origin was associated with shorter OS independently from Binet stage.**Summary.** Our analysis indicates the presence of relevant disparities between Japanese and European CLL patients. However, inter-observer variability and diverse techniques used for diagnostics could mimic differences which are not biologically or clinically reflected. These points require special attention. The presented data will serve as a basis to establish harmonized assessment by an independent panel. A prospective comparative study including cytogenetic and molecular analysis has already been initiated.

Table 1. Unadjusted and adjusted (according to Binet stage) comparison of Japanese and European CLL patients at diagnosis

Marker	Japanese (n analyzed)	European (n analyzed)	P-value unadj.	P-value adj.
Advanced Binet stage (B or C) (% patients)	33.3 (n=96)	18.8 (n=313)	**0.003**	-
Follow up (median, years)	5.1 (n=96)	6.2 (n=316)	**0.008**	**0.023**
Age at diagnosis (mean, years)	65.3 (n=96)	62.3 (n=316)	**0.020**	**0.017**
Male sex (% patients)	59.4 (n=96)	60.1 (n=316)	**0.895**	**0.904**
Hemoglobin (mean g/dl)	12.9 (n=96)	13.9 (n=284)	**<0.001**	**<0.001**
Platelets (mean number *10^4/µl)	16.9 (n=96)	20.1 (n=281)	**<0.001**	**<0.001**
Lymphocytes (mean number *10^3/µl)	26.3 (n=96)	24.6 (n=281)	**0.701**	**0.602**
High lactic dehydrogenase (% patients)	25.5 (n=94)	12.5 (n=306)	**0.001**	**<0.001**
CD23- (% patients)	33.8 (n=80)	2.3 (n=291)	**<0.001**	**<0.001**
CD38+ (% patients)	17.6 (n=51)	30.6 (n=291)	**0.059**	**0.056***
Treatment free survival				
All patients (median, years)	5.6 (n=95)	7.2 (n=314)	**0.100**	**0.466**
Typical CLL only (median, years)	5.6 (n=61)	7.2 (n=307)	**0.023**	**0.859**
Overall survival				
All patients (median, years)	21.5 (n=95)	22.9 (n=316)	**0.193**	**0.793**
Typical CLL only (median, years)	9.6 (n=61)	23.0 (n=308)	**0.003**	**0.023**

* Binet stage lost significance in the multivariable model

0740

THE EXPERIENCE OF ALLELE-SPECIFIC POLYMERASE CHAIN REACTION AND MULTICOLOR FLOW CYTOMETRY USE IN MINIMAL RESIDUAL DISEASE ASSESSMENT IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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The minimal residual disease (MRD) assessment was produced by means of multicolor flow cytometry and allele-specific polymerase chain reaction (PCR) in parallel for 45 peripheral blood specimens taken from 37 patients with B-cell chronic lymphocytic leukemia (B-CLL). Both methods results were similar in 93.3% (42 investigations of 45) with Spearman's rank correlation coefficient of 0.87 ($p < 0.0001$). 3 investigations of 45 (6.7%) have revealed malignant B-cells by one of the methods only: in first case by 6-color flow cytometry and in two others by real-time PCR.153 patients with B-CLL who have received combined FCR chemotherapy have undergone MRD assessment during the treatment period. The patients differed by gender - 56 female patients and 97 male ones, and by age: from 27 to 82 years. 290 investigations were produced in total: 222 of them were the analysis of peripheral blood specimens (98 investigations for interim evaluation of tumor clone, 98 - after 3 cycles of FCR chemotherapy and 124 investigations for final evaluation 6 cycles of chemotherapy after). The residual B-CLL population was performed using international standardized approach (Rawstron AC et. al, 2007; 21 (5): 956-64) which was modified for 5-color flow cytometry (Cytomics FC500, BC) and 6-color flow cytometry (FACS Canto II BD). 73 patients were undergone final MRD assessment with peripheral blood and bone marrow investigations. Of 124 B-CLL patients 65 (52.4%) went in immunophenotypical remission. In 16 (21.9%) cases the malignant B-CLL clone was determined in bone marrow only.In summary, the MRD assessment in B-CLL by means of multicolor flow cytometry has sufficient sensitivity and specificity and high correlation with real-time PCR results of MRD assessment using *patient-specific primers*. If MRD is not determined in peripheral blood, it is rational to investigate bone marrow for more exact evaluation of malignant cells elimination.

0741

CONTRADICTORY IMPACT OF SERUM BLYS LEVELS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), WALDESTRÖM'S MACROGLOBULINEMIA (WM) AND MULTIPLE MYELOMA (MM)

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Background. B-Lymphocyte-Stimulator (BLyS) is a member of the TNF-superfamily mainly produced by myeloid cells, monocytes, dendritic cells and osteoclasts in the bone marrow; it is involved in normal or malignant B cells' and plasmablasts' differentiation, proliferation, survival and in immunoglobulin production. It may be proteolytically cleaved from the cell membrane and circulate as a soluble protein. Few studies reported increased serum soluble BLyS in B-non Hodgkin lymphoma (B-NHL) and MM, especially in familial cases, but decreased in CLL while the clinical repercussions of BLyS levels remain unclear. **Aims.** To study the impact of serum BlyS levels on time to first treatment (TFT) and overall survival (OS) in patients with CLL, WM and MM. **Patients and Methods.** 203 patients were studied, of whom 73 had CLL, 33 WM and 97 MM. In the CLL cohort, 61% were males and 39% females and patients' median age was 60 years; 55% were in Binet stage A, 35% B and 10% C. CLL patients' median TFT was 34 months and median OS 79 months. Of WM patients, 76% were males and 24% females, median TFT was 6 months and median OS 103 months. In the MM series, 51% were males and 49% females; 24%, 33% and 43% of patients were in Durie and Salmon stage I, II and III respectively while 26%, 26% and 48% were in ISS stage 1, 2 and 3 respectively. Median TFT was 1 month and median OS 49.5 months. Sera drawn from patients at diagnosis were frozen and then, retrospectively, tested. Serum BLyS levels were determined by ELISA (R&D, Quantiquine) and assessed by Kaplan-Meier analysis, while survival curves were plotted and compared with the log-rank test. **Results.** Median serum BLys value was 65 pg/ml (range: undetectable - 680) in CLL, 218 pg/ml (range: 88 - 13200) in WM and 122 pg/mL (range: undetectable-921)in MM while it was 183 pg/mL (range: undetectable-381) in 14 healthy individuals. In CLL patients, serum BLys values below median were related to a significantly shorter TFT compared to values above median (p=0.0002) (Figure 1A) but no correlation was found with overall survival. In WM, there was not any correlation between BLyS levels and TFT or OS. In MM, serum BLyS levels above median correlated with a shorter OS (p=0.04, Figure 1B) but had no impact on TFT. **Conclusions.** The highest serum BLyS levels were observed in WM but did not affect TFT or OS. Low serum levels correlated with shorter TFT in CLL while, on the contrary, increased levels correlated with a shorter OS in MM. In view of the recent development of anti-BLyS agents, careful evaluation of its diverging effects in lymphoproliferative disorders is needed.

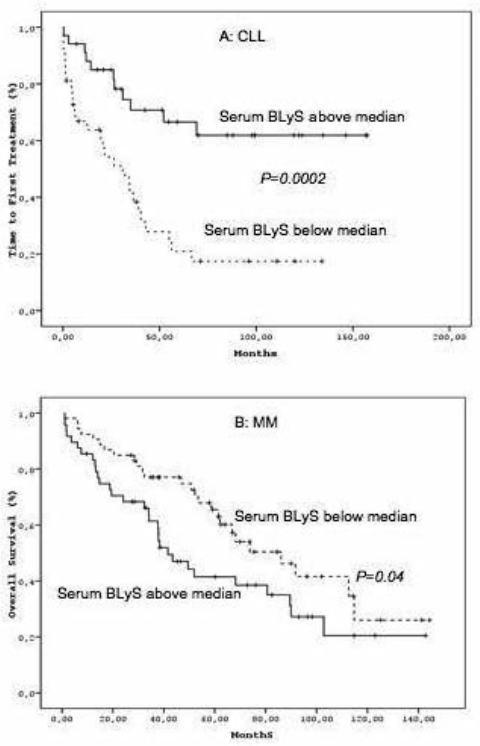

Figure 1.

0742

COMBINED ACTION OF NOVEL CD37 ANTIBODIES AND FLUDARABINE ON CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background. Chemo-immunotherapy involving the monoclonal antibody (mAb) rituximab represents the standard treatment for chronic lymphocytic leukemia (CLL). Due to known limitations of the CD20 and CD52 antibodies in current clinical use for treating CLL, we conducted an assessment on CLL lymphocytes *ex vivo* of the purine analog fludarabine in combination with two newly developed mouse-human chimeric and humanized antibodies to the tetraspanin CD37, mAb 37.1 and mAb 37.2, which had shown promising *in vitro* anti-CLL activity as single agents (Krause *et al.*, 2011, Leukemia). **Methods.** Treatment-induced phosphatidylserine exposure was examined in freshly isolated CLL cells after treatment with 10 µg/ml of mAbs and 3 or 10 µM of fludarabine. At these fixed concentrations CD37 antibodies were compared to rituximab and alemtuzumab as single agents and in combination with chemotherapeutic agents. The expected additive effects of binary combinations were calculated as fractional products of the separate single agent effects. **Results.** The previously observed high direct cell death induction by the CD37 antibodies was reproduced in an independent set of eight freshly isolated CLL samples with the highest induction of phosphatidylserine exposure by mAb37.1, followed by mAb37.2 and alemtuzumab, which in turn clearly surpassed that by rituximab. While treatment with mAbs led to similar induction of phosphatidylserine exposure after 24 or 48 hours, apoptosis induction by chemotherapeutic agents was much more pronounced after the longer treatment period. The mean apoptosis induction after 48 hours by mAb 37.1 and 10 µM fludarabine was ~ 50 % for both single agents, but reached ~ 90 % in combination. This indicated strong mutual enhancement and clearly surpassed the apoptosis induction by combi-

nations of the other antibodies with fludarabine at this concentration. Relative to untreated samples, the combination of 10 μM fludarabine with all antibodies tested led to significantly increased phosphatidylserine exposure according to paired Student's t-test. For all mAbs tested, the apoptosis induction by combinations with fludarabine significantly surpassed that by the antibodies alone. In contrast, only the combination with mAb37.1 induced apoptosis significantly more efficiently than fludarabine alone. **Conclusions.** In line with its remarkable capacity of inducing apoptosis in CLL cells, combinations of mAb 37.1 with fludarabine consistently resulted in higher percentages of apoptotic cells than correspondent combinations including the mAbs in current clinical use.

0743

THE ROLE OF IMAGING METHODS IN CHRONIC LYMPHOCYTIC LEUKEMIA: SIGNIFICANT INTERNAL LYMPHADENOPATHY IS FREQUENT AND ASSOCIATED WITH SHORTER OVERALL SURVIVAL

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Background. Chronic lymphocytic leukemia (CLL) is historically regarded as "liquid" malignancy but a significant subgroup of patients (pts) also presents with lymphadenopathy. With regard to extent of organomegaly, clinical staging systems by Rai and Binet rely solely on physical examination; the current version of NCI-WG guidelines for diagnosis and treatment of CLL does not recommend routine use of imaging methods in the management of CLL. However, several recent studies suggested that detection of internal lymphadenopathy may be associated with unfavourable prognosis. **Aims.** to evaluate the impact of internal lymphadenopathy on clinical course of pts with CLL. **Patients and Methods.** We performed a retrospective assessment of medical records from pts with CLL followed up at our institution between 2000-2010 who underwent a computer tomography (CT) of chest/abdomen or abdominal ultrasonography. A total of 301 pts were included (198 males, 103 females, median age, 63 years [range, 31-88]). Low/intermediate/high risk according to Rai modified staging was present in 38/50/12%. **Results.** During the course of CLL, internal lymphadenopathy was detected in 142 pts (48%): abdominal, 37%, mediastinal, 0.3%, abdominal and mediastinal, 11%. Bulky lymphadenopathy (defined as lymph nodes ≥ 5cm) developed during the course of the disease in 84 pts (28%). Interestingly, internal lymph nodes were frequently larger than palpable lymphadenopathy (45%; same size, 41%; external lymph nodes larger, 14%; n=117). Importantly, overall survival (OS) was significantly shorter in patients with internal lymphadenopathy (median 87 vs 124 months, p=0.024); there was also a trend towards shorter OS in pts with bulky lymph nodes (median 88 vs 112 months, p=n.s.). **Conclusions.** Our data strongly suggests that imaging methods should be incorporated into routine diagnostic workup of CLL as more than 51 % of pts had detectable internal lymphadenopathy during the course of the disease and 28% developed massive lymphadenopathy; in addition, patients with internal lymphadenopathy had shorter overall survival. These findings appear to have principal impact on the clinical management (decisions regarding therapy, adequate staging and assessment of therapeutic response, monitoring for relapse/progression). Updated results will be presented. Supported by Research Project MZO 00179906 from Ministry of Health, Czech Republic.

0744

A RETROSPECTIVE, SINGLE CENTRE, STUDY COMPARING CLINICAL AND LABORATORY FEATURES IN PATIENTS WITH ATYPICAL CD5 -VE CLL WITH THOSE FROM PATIENTS WITH STANDARD (CD5 +VE) CLL.

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Background. Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in developed countries. 7- 20% of cases do not show B cell expression of CD5 and are included in the atypical CLL group. CD5 is used in routine clinical practice to distinguish CLL and mantle cell lymphoma (MCL) from other types of lymphoproliferative disorders (LPDs). Confidently distinguishing CD5-ve CLL from other forms of LPDs can be challenging.DesignA retrospective analysis (2006-2010) was undertaken of patients diagnosed with CD5 -ve CLL with collection of clinical information and laboratory data including characterization of morphological, immunophenotypic and cytogenetic features and comparison with CD5 +ve cases diagnosed in the same five year period.Results239 cases of CLL were diagnosed during the study period from a catchment population of approximately 600,000. All cases fulfilled diagnostic criteria for CLL with peripheral blood lymphocyte counts of > 5x10^9/L. Biological prognostic factors including FISH analysis were available for 120 patients using probes specific to TP53 (17p13.1), ATM(11q22.3), centrometric region of chromosome 12 (12p11.1-q11) and d13S319(13q14.3). 55% of cases were male (median age 69.9 years, range 31-85), median age for females was 72.1 years (range 43-89). A total of 25.5% of cases were CD5 -ve (defined as <20% antigen expression on peripheral blood B cells) which is a slightly higher proportion than in other published series.57 % of patients with CD5 -ve CLL had CT scan at diagnosis to look for lymphoma.13 % had other diagnostic procedures performed, including bone marrow and/or lymph node biopsies. Baseline lymphocyte counts ranged between 5.3-174.8 x10^9/L with a similar distribution between CD5 +ve and CD5 -ve cases. With the exception of CD5 expression, flow cytometry cell profiles were identical in both groups. Bone marrow immunophenotyping, where available, showed identical profiles with peripheral blood in both CD5 +ve and CD5 -ve groups. Most of CD5 -ve cases were noted to have atypical cell morphology (larger cells or cleft nuclei on a background of otherwise typical CLL cells). Cytogenetics analysis was performed on 79 patients. 16.4% were CD5 -ve of which 56% had a poor prognostic karyotype, (45% 11q-, 0% 17p- and 11% both 11q- and 17p-). Of CD5 +ve CLL cases, 24% had poor prognosis karyotypes (13% 11q-, 9% 17p- and 2% both 11q- and 17p-). Median time from diagnosis to treatment was 12 months for CD5 -ve cases and 15.5 months for CD5 +ve cases.Conclusions This study demonstrated that CD5 -ve CLL is a relatively common occurrence in unselected haematology practice. CD5 +ve and CD5 -ve CLL were similar in terms of age, sex distribution and presenting lymphocyte counts. Cell phenotypes were similar in both groups except for levels of CD5 expression. Poor prognosis karyotypes were more prevalent in CD5 -ve CLL with a shorter time to first treatment.

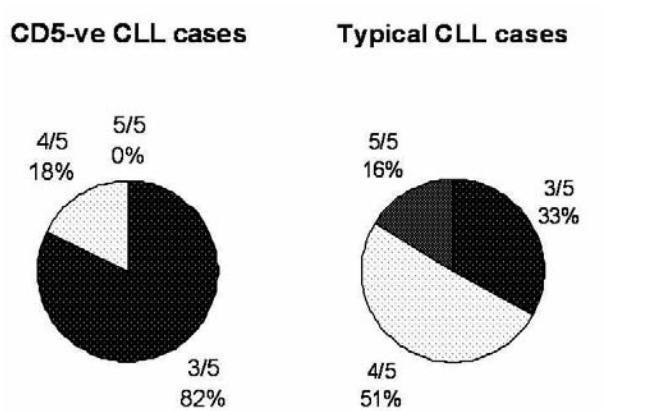

Figure 1. Patient groups stratified according to scoring criteria for CLL.

0745

THE PROGNOSTIC EVALUATION OF TCL-1, MCL-1, BCL-2, AND BAX EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background. Chronic lymphocytic leukemia is characterized with the accumulation of small mature-appearing CD5+, CD19+ and CD23+ lymphocytes in the bone marrow, blood, lymphoid tissues and very heterogeneous clinical course. Many adverse prognostic factors including the expression CD38, ZAP-70, Ig VH genes mutational status, lymphocyte doubling time have been described. **Aims.** In this study we aimed to investigate TCL-1, MCL-1, BCL-2 and BAX expression immuno histochemically in CLL patients and analyzed the prognostic value in the relationship between some other CLL prognostic markers such as clinical stage, ZAP-70, CD38, chromosomal abnormalities and infiltration of bone marrow pattern. **Methods.** 100 CLL patients, having undergone cytogenetic evaluation by FISH and 60 patients who showed normal bone marrow as a control group were included in this study. TCL-1,MCL-1,BCL-2,BAX were stained by automated methods (VENTANA,Benchmark XT). In the evaluation of TCL-1 and BAX staining , diffuse cytoplasmic staining of more than 20% of neoplastic B cells was accepted as positive. In the evaluation of MCL-1 and BCL-2 staining, diffuse cytoplasmic and nuclear staining of more than 20% of neoplastic B cells were accepted as positive. **Results.** In the immunohistochemistry section staining TCL-1 was detected in 70 patients (70%), BCL-2 was detected in 58 patients (58%), positive BAX was detected in 61 patients (61%). Positive MCL-1 was not observed in any patients. and control group showed no positive TCL-1, MCL-1, BCL-2, BAX expressiin. Cytogenetic abnormalities were observed in 41 patients group (41%). 15-year- event free survival of 42 patients with Rai stage 0-1 was found to be 184,4 months. 15-year- event free survival of 37 patients with Rai stage 2 was found to be 81,1 months (p=0.003). 15-year- event free survival of 62 patients with positive. TCL-1 was 124,3 months while 15-year- event free survival of 17 patients with negative TCL-1 was 74% (p=0.0043). In the univariate analysis age, stage (RAI 0-I-II-III) ,type of bone marrow stiffness, CD 38, TCL-1 remained significant while in the multivariable analysis (Cox proportional hazard model backward) stage (RAI 0-I-II-III), type of bone marrow stiffness and nodular diffuse (p=0.019, RR=3.055) CD38 (p=0.001, RR=0.228) remained significant. **Conclusions.** As a result , TCL-1 is accepted as a prognostic marker and may show progression of the disease follow up in CLL patients.. Inhibition of TCl-1 and especially in B-cell receptor inhibitiory mechanisms and the drugs may change clinical progression of CLL patients showing expression of TCL-1.

0746

LOW DOSE ALEMTUZUMAB-ASSOCIATED IMMUNE THROMBOCYTOPENIA IN CHRONIC LYMPHOCYTIC LEUKEMIA

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The occurrence of alemtuzumab-associated immune thrombocytopenia (ITP) have been previously anecdotally reported in chronic lymphocytic leukemia (CLL) patients. Despite ITP is a common autoimmune complication in CLL, occurring in up to 5% of patients, it has never been reported as a significant event complicating alemtuzumab treatment in clinical trials. Recently, it has been reported a new distinctive form of secondary ITP occurring in 6 out of 215 patients with relapsing-remitting multiple sclerosis treated with alemtuzumab at very low-dose within the context of a randomized, controlled phase II trial (Cuker *et al*, Blood 2011).We investigated the incidence of ITP in a cohort of 64 consecutive patients treated at our center for relapsed/refractory CLL from 2003 to 2010 with low-dose alemtuzumab (Cortelezzi *et al*, Leukemia 2009). Alemtuzumab was administered at a dose that is one third weekly (30 vs 90 mg) and half cumulative (540 vs 1080 mg) with respect to the conventional schedule. ITP was diagnosed in 3 patients (4.7%) before alemtuzumab treatment during a median observation time of 76 months (range 6-280) and in 9 patients (14.8%) after alemtuzumab, during a median observation time of 30 months (range 2-93). ITP developed after a median time from alemtuzumab exposure of 12 months (range 1-42). Concomitant hemolytic anemia (Evans syndrome) was observed in one patient. At ITP diagnosis, median platelet count was $11x10^9$/L (range 3-70). Anti-platelet antibodies (Capture P® Method, ImmucorGamma, Norcross GA, USA) were found in 7 of the 8 patients tested. Only two patients showed a clinically significant bleeding and no patient suffered of severe bleeding events. Of the nine patients developing ITP after alemtuzumab, three progressed to a generalized disease after 3, 4 and 13 months respectively, and were treated with chemo-immunotherapy - one patient achieved a partial remission with ITP resolution, while the other two were treatment refractory and died in progression. In the remaining six patients, ITP was not associated to disease progression and they were treated with corticosteroids with or without intravenous immunoglobulins. Five patients achieved a complete remission, while one patient did not respond. Table 1 summarizes the clinical characteristics of the patients.In our cohort of CLL patients treated with alemtuzumab, the incidence of ITP was striking with 5.7 events/100 patient-year, that is almost three times higher than previously reported in CLLIn conclusion, we report for the first time the association between low-dose alemtuzumab treatment and ITP in a prospective cohort of CLL patients. This data suggested an important role of alemtuzumab induced disregulation of T-lymphocytes in the pathogenesis of ITP. As low-dose alemtuzumab have been advocated by our group to be an effective and less toxic treatment for CLL, and a retrospective practice-based study recently supported this notion, we would suggest to maintain a high level of vigilance and to consider routine monitoring for ITP in patients treated with this agent at low-dose.

0747

HAIRY-CELL LEUKEMIA: CHARACTERISTICS AND LONG TERM FOLLOW UP OF 57 PATIENTS

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Background. Hairy cell leukemia (HCL) is an uncommon, indolent, chronic B-cell lymphoproliferative disorder involving mainly bone marrow and spleen. Despite an usually indolent course, most patients require treatment for cytopenia or massive splenomegaly. Treatment has been revolutionized by the advent of interferon (IFN) and purine analogs. **Aims.** Few large series are described in the literature. In this long follow-up study, we describe presentation and evolution of HCL, particularly response to treatment and survival. **Methods.** All consecutive patients diagnosed with HCL between 1978 and 2011 in two Hospitals were included in this retrospective study. The criteria for response were those of the Consensus Resolution. Complete Response (CR) was defined as a morphological absence of Hairy Cells (HC) in the blood and bone marrow, without organomegaly, and normalization of cytopenias. A partial response (PR) required normalization of peripheral counts, together with at least 50% reduction in organomegaly and bone marrow HC, and <5% circulating HC. All other responses were considered as non responses. **Results.** Fifty seven patients were included with a median follow-up of 69.5 months (1-329). Two patients were diagnosed with HCL variant. Median age at diagnosis was 57 years (25-92). There was 44 male and 13 female (sex ratio M/F: 3.4).HCL was revealed by systematic blood count in 33 patients, by general symptoms, splenomegaly or infectious complications in 8, 4, and 9 patients, respectively.At diagnosis, 35 patients were symptomatic, with splenomegaly (n=33), hepatomegaly (n=10), and peripheral lymphadenopathies (n=7). Anemia (<10g/dL), thrombocytopenia (<100G/L), neutropenia (<1G/L) and monocytopenia (<0.1G/L) were present in 10, 48, 21 and 17 patients, respectively.Treatment was begun at diagnosis for 46 patients and delayed for 9 patients with a median time without treatment of 8 months (2-48).First line treatment choice were cladribine, pentostatin, IFN, rituximab and splenectomy in 19, 15, 15, 4 and 4 patients, respectively. Complete response (CR) rate, relapse rate and median relapse free survival (RFS) were respectively: cladribine (100%, 19%, 41.5 months), pentostatin (100%, 20%, 32 months), IFN (80%, 80%, 22 months), Rituximab (80%, 56%, 45.5 months) and splenectomy (50%, 75%, 8 months)Second line treatment were pentostatin (n=11), cladribine (n=8), IFN (n=8) and rituximab (n=3), with respective CR rates of 63%, 75%, 25% and 100%, relapse rate of 9%, 12,5%,85% and 33%, and median RFS of 28,19,20 and 35 months.13 patients received third line treatments and 4 a fourth one.26 patients had infectious diseases after treatment, and 7 developed secondary cancer.At the end of the study, 52 patients were alive, none died because of the disease. 39 were in CR, 6 in PR, and 7 with relapsed disease. 10 years estimated EFS and OS rates were 82% and 90%, respectively. **Conclusions.** This study confirms the good prognosis of this disease and previous observations that purine analogues induce long-term CR, included in second or more line of treatment. Outcomes for patients with recurrent disease have improved with the addition of rituximab to either purine analog with moderate infectious complications due to the immunosuppressive effects.

0748

CLINICAL FATE OF HIGH RISK CHRONIC LYMPHOCYTIC LEUKAEMIA (HR-CLL) PATIENTS TREATED WITH ALEMTUZUMAB BASED THERAPY

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Background. Treatment options for HR-CLL are limited. Alemtuzumab based treatment has been shown to be effective with variable response rates in clinical trials. However, the clinical fate of this group of patients is not studied before. Furthermore delivering this treatment poses a challenge in the day-to-day clinical setting outside trials due to 'unselected' patient cohorts with other co-morbidities. **Aims.** To determine the clinical course of HR-CLL treated with Alemtuzumab based therapy. **Methods.** A retrospective analysis of 51 HR-CLL patients treated at the Royal Marsden Hospital (London, UK) from January 2005 to December 2011. **Results.** Median age at the time of therapy was 60 (range 35-78; > 25% age >65), Charlson co-morbidity index range 0-6, and ECOG PS range 0-2. Indications for Alemtuzumab based therapy included *TP53* deletion at diagnosis (17%) or at relapse (33%) or Fludarabine-refractory or relapsed disease without *TP53* deletion (50%) or a combination of the above. 16 patients received Alemtuzumab alone; 35 received Alemtuzumab in combination with methyl prednisolone. Response was assessed according to NCI-WG criteria.OR 82% with CR rate 37% in the whole cohort. There was no significant difference in OR or CR according to the age groups (<>65 years), the line of treatment, *TP53* status or Alemtuzumab alone compared to Alemtuzumab and Methyl Prednisolone group (P = 0.25 and 0.32; 0.56 and 0.24; 0.14 and 0.38; 1 and 1 respectively). There was a difference in CR but not OR for patients treated within clinical trials compared to patients treated outside clinical trials (P = 0. 01 and 0.7).Common complications included bacterial infections (31%), and CMV reactivation (19%). Two patients had invasive fungal infections; one case each had EBV and adenovirus infections. Treatment was interrupted before the planned end date in 13% of patients. There were 2 treatment related deaths. There was no difference in tolerability observed or treatment discontinuation in patients <> 65 years.On the interim analysis of survival data with median follow-up of 3.6 years since therapy, 32 patients are alive; 14 had allogeneic HSCT and 19 had another line of treatment but did not receive HSCT. Ten had Alemtuzumab as subsequent treatment at relapse and had an OR of 77%. The median OS of the whole cohort was 47 months; the median OS for patients who had transplants was 26 months. Sub group survival data analysis is in progress. **Summary and Conclusions.** Alemtuzumab based therapy is highly effective in relapsed high risk CLL irrespective of *TP53* deletional status. However duration of therapy is relatively short lived with majority of patients needing another line of therapy. Alemtuzumab can be used repeatedly with good OR. Alemtuzumab based therapy is deliverable in routine clinical practice even to older patients with co-morbidities with comparable response rates and response durations as reported in clinical trials. There is a high risk for infections but this is comparable to other regimens used in relapsed refractory setting.

0749

DASATINIB PLUS FLUDARABINE IN PATIENTS WITH REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS OF A MULTICENTER PHASE II STUDY

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Patients with chemorefractory chronic lymphocytic leukemia (CLL) have an extremely poor prognosis. Development of chemoresistance in CLL is at least partly mediated by protective stimuli within the lymph node (LN) microenvironment. Dasatinib has activity against multiple kinases reported to be activated by the microenvironment including SRC, c-Abl and BTK. We recently showed by *in vitro* studies that dasatinib effectively inhibited the anti-apoptotic program and as a consequence, restored fludarabine sensitivity (Blood 2008;112:5141). These data indicate that CLL cells in chemoresistant niches may be sensitive to therapeutic strategies that include both dasatinib and purine-analogues.**Methods and Objectives:** We conducted an open-label phase 2 trial of Dasatinib/fludarabine combination in fludarabine-refractory CLL patients. Patients were treated with dasatinib 100mg once daily for 28 days. In patients that did not reach at least a PR, dasatinib was combined with oral fludarabine (40 mg/m^2 for 3 consecutive days q28) for at least 2 and maximally 6 cycles. The primary endpoints were response (IWCLL'08 criteria) and toxicity. CT-scans were performed at baseline, following dasatinib monotherapy, after 2 cycles of combination therapy and at end of protocol. At multiple time points peripheral blood-derived CLL samples and in a limited number LN biopsies were obtained. **Results.** The study was open at 3 sites in theNetherlands (Academic Medical Center Amsterdam, University Medical Center Groningen and Erasmus Medical Center Rotterdam). At time of analysis, 20 patients had been registered of which 18 patients received at least 1 treatment cycle. The median age was 69,5 years (range 29-82 years). Six patients (33%) had del (11q;22-23) and 2 patients (11%) haddel (17p;13). Patients had received a median of 5 prior treatment regimens (range 2-9). Eleven patients (61%) had bulky lymphadenopathy. PR was not observed following one cycle of dasatinib monotherapy. All but two patients (one Richter transformation and one patient refusal) continued for dasatinib/fludarabine combination treatment. At time of analysis, 3 patients completed all 6 cycles. Reasons for early discontinuation were: progressive disease (n=9), dasatinib related toxicity (n=2; both pleural effusion grade IV) and patient refusal (n=2). Fifteen patients had reductions in lymph node size. The mean maximal reduction in lymph node size was 30% (range 5%-79%; figure1). Three patients (17%) reached a PR. Twelve patients (67%) had stable disease as best response and 3 patients had progressive disease after 2 cycles of dasatinib+fludarabine. At time of analysis PFS following combination treatment was 11 months and OS was 17 months. Two patients proceeded to allogeneic stem cell transplantation. Numbers of SAE were 19; fever with/without proven infections being the most frequently reported (68%). Only 1 patient developed treatment related pleural effusion. There were no treatment related deaths.Currently, correlative ex vivo biological studies focussing on apoptosis regulation are being performed. **Conclusions.** In heavily pretreated fludarabine refractory CLL patients dasatinib/fludarabine combination treatment showed modest activity, notably as to LN size. As expected, infections were the most common toxicity. Dasatinib was relatively well tolerated. Future studies involving combinations of dasatinib at earlier phases of the disease should be con

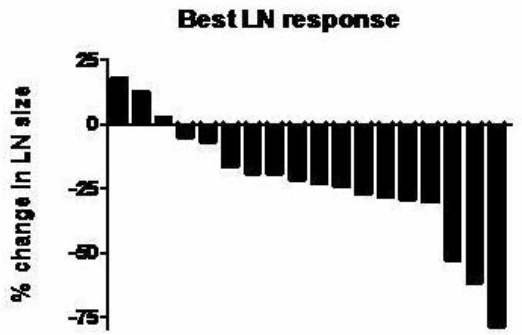

Figure 1.

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0750

ALEMTUZUMAB FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA - UPDATE OF A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background. Chronic lymphocytic leukaemia (CLL) accounts for 25% of all leukemias and is the most common lymphoid malignancy in Western countries. Comparatively new therapeutic options are monoclonal antibodies like alemtuzumab and rituximab administered alone or added to chemotherapy. However, the impact of these agents remains unclear, as there are hints of an increased risk of severe infections.**Aims**To assess the efficacy and safety of alemtuzumab compared to no further therapy or to other anti-leukaemic therapy in patients with CLL. **Methods.** We performed a systematic review with meta-analyses of randomized controlled trials (RCTs). We searched CENTRAL, MEDLINE, and EMBASE as well as conference proceedings from 01.1985 to 01.2012. Two review authors independently screened search results, extracted data and assessed quality of trials. **Results.** Of 1589 potentially relevant references, we included six RCTs involving 845 patients. Overall, we judged the quality of the trials to be moderate.Three trials (N=618) assessed the efficacy of alemtuzumab compared with no further therapy. Only one trial (N=335) reported overall survival (OS) with a statistically significant advantage for those patients receiving alemtuzumab (HR=0.65 (95% confi-

dence interval (CI) 0.45 to 0.94; P=0.021). The complete response rate (CRR; 3 trials) (RR=1.45; 95% CI 1.15 to 1.83; P=0.002) and progression-free survival (PFS; 2 trials) (HR=0.58; 95% CI 0.44 to 0.76; P<0.0001) were statistically significantly increased under therapy with alemtuzumab. However, a statistically significant 10-fold higher rate of cytomegalovirus (CMV) reactivation (RR=10.52; 95% CI 1.42 to 77.68; P=0.02) and infections (RR=1.32; 95% CI 1.01 to 1.74; P=0.04) occurred in patients receiving alemtuzumab.Two trials (N=177), evaluated alemtuzumab versus rituximab. Both studies did not report OS or PFS. We could not detect a statistically significant difference for CRR (RR=0.85; 95% CI 0.67 to 1.08; P=0.18) or treatment-related mortality (RR=3.20; 95% CI 0.66 to 15.50; P=0.15) between both arms, but a trend favouring the rituximab arm. One trial was stopped early due to an increase in mortality in the alemtuzumab arm. More serious adverse events occurred in this arm (43% versus 22% (rituximab), P=0.006).One trial (N=297), assessed the efficacy of alemtuzumab compared with chemotherapy (chlorambucil). Median survival has not yet been reached, 84% of patients were alive in each arm at the last follow-up date (24.6 months). Alemtuzumab statistically significantly improves PFS (HR=0.58; 95% CI 0.43 to 0.77; P=0.0001) compared with chlorambucil. Statistically significantly more asymptomatic (51.7% versus 7.4%) and symptomatic CMV infections (15.4% versus 0%) occurred in the patients treated with alemtuzumab. **Summary and Conclusions.** In summary, five of six trials reported an increased risk for infections in general and CMV reactivations.The currently available evidence suggests an OS, CRR and PFS benefit for alemtuzumab compared with no further therapy.The role of alemtuzumab versus rituximab still remains unclear. Further trials with longer follow-up are needed to evaluate the effects of both agents compared with each other.In comparison with chlorambucil, alemtuzumab seems to be favourable in terms of PFS, but a longer follow-up period is needed to determine whether this effect will translate into a survival advantage.

0751

RITUXIMAB FOR CHRONIC LYMPHOCYTIC LEUKEMIA: A SYSTEMATIC REVIEW WITH META-ANALYSIS

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Background. Chronic lymphocytic leukemia (CLL) is the most common lymphoid malignancy in Western countries. The administration of rituximab in addition to chemotherapy in the treatment of CLL patients showed promising results, but the effects differ between studies. Therefore, the impact of this monoclonal antibody remains uncertain. **Aims.** This systematic review with meta-analysis assessed the efficacy and safety of chemotherapy plus rituximab compared to chemotherapy alone in patients with CLL. **Methods.** We performed a systematic review with meta-analysis of randomized controlled trials (RCTs). Therefore we searched CENTRAL, MEDLINE, and EMBASE as well as conference proceedings from January 1985 to January 2012 for RCTs. Two review authors independently screened search results, extracted data and assessed quality of trials. **Results.** A total of 1150 potentially relevant references were screened. We included three RCTs (N=1421 patients). The quality of these three trials ranged from moderate to high. The meta-analyses showed a statistically significant OS and PFS advantage for patients receiving rituximab (OS: HR 0.78 95% confidence interval (CI) 0.62 to 0.98, P=0.03; PFS: HR 0.64 (95% CI 0.55 to 0.74, P < 0.00001). Rituximab caused more AEs, WHO grade 3 or 4 (RR 1.15, 95% CI 1.08 to 1.23, P < 0.0001). There was no statistically significant difference regarding treatment-related mortality (RR 1.19, 95% CI 0.70 to 2.01, P = 0.52). **Summary and Conclusions.** The available evidence showed that patients receiving additional rituximab benefit in terms of OS and PFS compared to those with chemotherapy alone. On the other hand, additional treatment with rituximab increased the amount of occurring severe acute adverse events, but this did not lead to a statistically significant difference in the treatment-related mortality.

0752

EFFICACY OF COMBINATION FLUDARABINE AND ALEMTUZUMAB IN PATIENTS WITH RELAPSED AND REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. It is known that refractory to the fludarabine-containing regimens and their combination with rituximab is observed in some patients with chronic lymphocytic leukemia (CLL). The possibility of use of alemtuzumab in clinical practice has become a new milestone therapy relapse and refractory CLL. **Aims**. To study the efficiency and toxicity of the combination of fludarabine and alemtuzumab (FluCam) in patients with relapsed and refractory CLL. *Methods.* Twenty-four CLL patients aged 36 to 69 years (median 56 years) were included in the study. Fourteen patients were in Binet stage B, and 10 in stage C. The refractory to prior therapy was registered in 9 (38%) patients, 15 (62%) patients had relapsed CLL. The prior therapy included alkylating agents and fludarabine-containing regimens (FC, FCM, and FCR). ZAP-70 and CD38 expression was positive by flow cytometry in 92% and 67% of cases, respectively. Deletion 17p was determined by FISH analysis in 38% of patients, complex chromosomal aberrations were detected in 29%. The program FluCam consisted of fludarabine (25 mg/m^2 intravenously, days 1-3), and alemtuzumab (30 mg subcutaneously, days 1-3, after a standard dose escalation). Courses repeated every 28 days, their numbers ranged from 4 to 6. Throughout the period of treatment and 2 months after it pneumocystis pneumonia prophylaxis with trimethoprim/sulfamethoxazole 960 mg orally once daily, 3 times a week was carried out, as well as reactivation of cytomegalovirus infection valganciclovir 900 mg (two 450mg tablets) orally once daily was accomplished. Pegfilgrastim 6 mg subcutaneously was administered on day 6 of each course of therapy as primary prophylaxis for neutropenia. DNA of cytomegalovirus, Epstein-Barr virus, herpes types 1 and 2, hepatitis B and HCV RNA were monitored in all patients before treatment and every 2 weeks during therapy with polymerase chain reaction. **Results**. By using combination FluCam as a 2-4 line therapy an overall response was obtained in 18 (75%) patients, complete remission (CR) was achieved in 6 (25%) patients, and partial remission (PR) - in 12 (50%) of cases. Disease progression was observed in 2 (8%), stabilization of the process - in 4 (17%) patients. Among 6 patients with CR minimal residual disease was not revealed in 4 patients. Median follow-up was 23 months. The median overall survival was not reached. The median progression free survival (PFS) was 11 months (95% CI: 8.2-17.6) in the overall group of patients. The median PFS was 21 months in patients with CR, among patients with PR - 8 months. A direct correlation between PFS was determined in patients with the presence of complex chromosomal aberrations and deletion 17p (R=0.624; p=0.017). The most common manifestations of hematologic toxicity were neutropenia (58%), thrombocytopenia (21%) and anemia (17%). Infectious complications were occurred in 54% of patients, including severe ones - in 17%, in the form of pneumonia. Asymptomatic CMV reactivation was revealed in 42% of cases. All side effects were curable. **Conclusions**. The combination FluCam is highly effective, characterized by acceptable toxicity in patients with relapsed and refractory CLL.

0753

SEVERE INFUSION-RELATED REACTIONS ARE UNCOMMON IN RITUXIMAB-TREATED CLL PATIENTS: RESULTS FROM A NATIONAL OBSERVATIONAL STUDY

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Since the approval of rituximab (R), there have been concerns about infusion-related adverse reactions, especially in patients with a large number of circulating tumor cells as in chronic lymphocytic leukemia (CLL). In the randomized CLL8 trial by the German CLL study group, comparing R-FC with FC, relatively few serious infusion-related adverse events were noted, but this has not been studied in a general population. We therefore conducted a multi-center observational trial to study the incidence and management of infusion-related adverse events.Patients with CLL requiring therapy were eligible for this study after informed consent if the planned therapy contained rituximab. Therapeutic regimen, premedication and management of adverse events were up to the physician and not stated in the protocol.Totally 96 (70 males, 26 females) patients from 19 centers were enrolled. Median age was 67 years (range 41-89). Median time from diagnosis to study inclusion was 3.4 years (range 0-17). Previous therapy for CLL had been given to 46 patients. Five patients had previously received rituximab. Median leukocyte count before therapy was $70*10^9$/L (range 2-422).The most used regimens were standard (n=66) or reduced (n=12) R-FC. Other regimens were R-bendamustine (n=8), R-CHOP (n=2), R-COP (n=3), R-chlorambucil (n=1), R-cyclophosphamide (n=1), R-fludarabine (n=1) and rituximab monotherapy (n=2). Of the 96 patients 81 received rituximab from the first course of therapy, nine from cycle 2, two from cycle 3, three from cycle 4 and one from cycle 5. The rituximab infusion was split over two days with 100 mg given the first day and the remaining dose at day 2 during the first course in seven patients and during the second in two patients. Almost all patients (98%) received antihistamine. and most of them also paracetamol (88%) and corticosteroids (86%) as premedication before the first rituximab infusion.Six courses of therapy were completed in 46 (48%) patients. Reasons for premature discontinuation include therapy-related toxicity (n=23), good clinical response after fewer courses (n=14), decision of the patient (n=4), refractory disease (n=4), secondary malignancy (n=1), death (n=1), stem cell transplantation (n=1), change of hospital (n=1) or not stated (n=1).Adverse reactions (AR) during rituximab infusion were seen in 56 patients (58%). However, grade 3 (n=5) or 4 (n=1) reactions occurred in only five patients on six occasions and in all but one case only during the first course. The reactions consisted of rigors (n=2), nausea (n=1), hypotension (n=1), dyspnea (n=1) or flushing (n=1). Only two of these patients had a leukocyte count > $50*10^9$/L. The AR was treated with steroids in 29 patients and pethidine was used in 7 cases. The patient with grade 4 hypotension was also given atropine. No cases of tumor lysis syndrome were recorded despite that the median reduction of leukocyte count between the first and second course was 91%.In this study the number of severe rituximab-related infusion reactions was low despite a high number of circulating tumor cells. Thus, R-FC or other rituximab-containing regimens can be considered safe in the general population of CLL patients.

0754

EFFICACY AND TOLERANCE OF FLUDARABINE AND CYCLOPHOSPHAMIDE(FC) COMBINATION REGIMEN IN ADVANCE STAGE CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukemia (CLL) is the classical leukemia of the elderly and the treatment often must be tailored to the patient's fitness level and ability to tolerate more toxic combination therapies.Many randomized trials have shown that the addition of cyclophasphamide to fludrabine clearly improves the CR and OR rate and PFS as compared with fludarabine monotherapy. An additional important result of these trials was that FC did not increase the rate of severe infections despite inducing more grade 3 and 4 neutropenias.aimsto assess the efficacy and tolerability of FC combination chemotherapy in Iraqi adult patients with advanced chronic lymphocytic leukemia, reflected by response rate, treatment free survival and overall survival.methodsA prospective study carried out in Baghdad teaching hospital and national hematology center in Baghdad between February 2005 and fabruary 2009 included 64 Iraqi patients aged between 39-77years old with advanced stage CLL. A written informed consent was obtained from all patient prior to start of therapy.they received FC combination therapy (fludarabine 25 mg/m^2 plus cyclophosphamide250mg/m^2 for 3 days intravenously, repeated every 28 days). Treatment was administered for 6 courses.ResultsOut of 64 CLL patients with Binet stage B&C who were included in this study, 41(64.1%)patients were male and 23(35.9%)patients were females (M:F ratio1.7:1) with median time of follow up 26 months. median age was 59.9years. forty- eight (75%)patients were previously untreated, of them 22(45.8%)patients were stage B and 26(45.1%)patients were stage C while the other 16(25%)patients with CLL were previously treated with an alkylating agents, of them7(43.7%) of stage B and 9(56.2%) with stage C.This combination chemotherapy resulted in 39.1% complete remission and 39.1% partial remission.The 2 years median treatment-free survival was 90% with the median duration of response was 18 months. there was a significant difference ($p<0.005$) between the different Binet stage group(C&B) and degree of response, with better response rate in those with stage C than those with stage B. the overall response rate was 88.3%, 65.5% for stage C and B respectively.The major toxicity(grade 3-4) were nausea and vomiting while the myelosupression of grade 1&2 for leucopenia and neutropenia occur in 19%, 14% respectively conclusion Fludarabine and cyclophosphamide combination regimen is an effective therapy for patients with advanced CLL with high response and complete remission rate in both untreated and previously treated CLL patients, with good tolerability to this combination

0755

EPHB4/ RAC1/CDC42/RHOA PATHWAY CONTRIBUTES TO IMATINIB RESISTANCE IN CHRONIC MYELOID LEUKEMIA

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Background. Although the mechanism of imatinib (IM) resistance in chronic myelogenous leukemia (CML) has been intensively investigated, few studies have investigated the role of Eph receptors in IM resistance. In 2003, Ohmine et al suggested the disturbance of RhoA protein kinase signaling, was involved in imatinib resistance. In 2010, Suzuki et al reported that there existed a new mechanism of IM resistance mediated by the activation of Ras/MAPK pathway and EphB4 in Philadelphia chromosome-positive acute lymphocytic leukemia (ALL). **Aims.** Our purpose was to address the role of EphB4 in IM resistance. Then, in order to further determine the mechanism of EphB4 receptor contributing to IM resistance, we detected a serious of phosphorylation of Ras/MAPK and RhoA related signaling proteins. **Methods.** K562 IM-resistant cell lines (K562-R) were supplemented with 5.45 mg/L IM. The stable underexpressing EphB4 cells (K562-R-EphB4-sh) were obtained (Oligonucleotides for shRNA were designed using ABI online software, Lentiviruses were generated and were cotransfected in 293T cells, then directly infected K562-R cells). Semi-quantitative PCR (SQ-PCR) was performed on ABI 9700 system. Phosphorylation of molecules was analyzed by Western Blot method. BALB/C female nude mice (4-5 weeks of age) were used in xenograft experiments. **Results**. Western Blot analysis showed that the EphB4 receptor was overexpressed in the IM-resistant K562-R cell line (P<0.001). We established the stable underexpressing EphB4 cell line K562-R-EphB4-sh. MTT analysis suggested that K562-R-EphB4-sh cell was sensitive to IM (IC50 0.93 mg/L) and K562-R showed IM resistance (IC50 5.45 mg/L, P<0.001). In a xenograft model, K562-R tumor volumes significantly increased in the setting of IM treatment (2301.25 mm^3 versus 1733.82 mm^3, P=0.001). In contrast, the K562-R-EphB4-sh xenograft volumes had no definite change (1630.16 ± 412.01 mm^3 versus 1720.06 ± 290.54 mm^3, P=0.40) after receiving IM treatment. Overall survival after 30-treatment-days was 66.7%, 0% and 33.3% in the K562-W, K562-R and K562-R-EphB4-sh xenograft groups, respectively (P=0.001). Meanwhile, there were no differences of the phosphorylation of MEK/ERK between K562-R and K562-R-EphB4-sh cells or xenograft tissue. Nevertheless, phosphorylations of RhoA and rac1+cdc42 were significantly decreased in K562-R-EphB4-sh cells and xenograft tissue compared with K562-R cells and xenograft tissue (P<0.001). **Conclusions**. We confirmed that EphB4 expression was increased in IM resistant cells and decreased in K562-R-EphB4-sh cells conferred sensitivity to IM. Similarly, low-expression of EphB4 also became sensitivity to IM in K562 xenograft model. MEK/ERK activity was not change with down-regulated EphB4. The phosphorylations of RhoA and Rac1+cdc42 were simultaneously decreased in K562-R-EphB4-sh cell line and xenograft tissue comparing with K562-R cell line and xenograft tissue. These data supported a new marker of IM resistance mediated by the activation of EphB4/Rac1/cdc42/RhoA pathway in CML.

0756

SMALL CLONES OF BONE MARROW CELLS WITH BCR-ABL GENE AMPLIFICATION PREDICT UNFAVORABLE OUTCOME OF IMATINIB CML TREATMENT

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Background. The therapy of CML by TKI have shown impressive results last decade. However 24% of patients (pts) on imatinib therapy have failed to achieve optimal response in 18 months (O'Brien et al. , 2003) and 10% pts have lost the achieved response in 5 years (Druker BJ et al. , 2008). The amplification of BCR-ABL gene have postulated in a lot of review papers as one of the important mechanism of resistance to TKI therapy. Nevertheless there is no any large cohort of CML patients (pts) data according to the role of BCR-ABL gene amplification as well as the significance of clone size of BM cells with amplification in resistance to TKI. **The purpose** of this study was to elucidate the predictive significanse of BCR-ABL gene amplification and the role of small clones of BM cells with BCR-ABL gene amplification in large cohort of CML patients in resistance to imatinib. **Materials and Methods.** 174 CML patients (130 -CP, 44 -AP) on imatinib 400 or 600 mg QD therapy more than 12 months duration were included in study**.** According to the ELN criteria (2009) 51 pts have achieved optimal or suboptimal response, 86 pts have failure and 37 pts have loss of response. BM samples were analyzed by cytogenetic and D-FISH analysis with dual color/dual fusion BCR-ABL gene probe ("Vysis") with cutoff <1%. **Results.** In our study BCR-ABL gene amplification was revealed in 44/174 patients (25%). From 44 pts with amplification 28(63,6%) pts were in CP and 16(36,4%) - in AP (p<0.05). FISH did not revealed BCR-ABL amplifications in pts with optimal and suboptimal responses. Additional copies of BCR-ABL gene (from 1 to 7) were found in 32 (37,2%) pts with failure and in 12 (32,4%) pts with loss of response (p>0,5). The probability of complete cytogenetic response (CCyR) achievement in pts with BCR-ABL amplification was significantly lower than in pts without it (31,6% vs. 63,8%, p<0,0001). The probability of CCyR achievements in pts of Q1-Q2 (1-6% BM cells with BCR-ABL amplification) did not differ from pts of Q3-Q4 (7-72% BM cells with BCR-ABL amplification) (p>0,5). **In conclusion**, BCR-ABL gene amplification is not rare in resistant patients in chronic and accelerated phases of CML. The pts with low level clones with BCR-ABL amplification have the same unfavorable prognosis as well as pts with high level clones.

0757

EFFICACY AND SAFETY OF BOSUTINIB FOR PHILADELPHIA CHROMOSOME-POSITIVE LEUKEMIA IN OLDER VERSUS YOUNGER PATIENTS

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Background. Bosutinib is an oral dual Src/Abl kinase inhibitor with potent activity in Bcr-Abl-positive leukemia. **Aims.** To evaluate the efficacy and safety of bosutinib in older (≥65 years; n=119) and younger (<65 years; n=451) patients. **Methods.** Bosutinib 500 mg/day was administered to patients in 3 cohorts: chronic phase chronic myeloid leukemia (CP CML) after imatinib (CP2L cohort; n=287); CP CML after imatinib + dasatinib and/or nilotinib (CP3L cohort; n=119); and accelerated/blast phase (AP/BP) CML or acute lymphoblastic leukemia after imatinib ± dasatinib and/or nilotinib (ADV cohort; n=164).

	CP2L cohort		CP3L cohort		ADV cohort	
	≥65 y	<65 y	≥65 y	<65 y	≥65 y	<65 y
n[a]	63	223	25	92	29	122
MHR	–	–	–	–	28%	30%
CHR	81%	87%	72%	74%	14%	25%
2-y probability of maintaining CHR[b]	67%	75%	65%	69%	75%	54%
n[a]	61	204	22	88	26	116
MCyR	43%	57%	27%	34%	23%	32%
CCyR	38%	45%	23%	24%	19%	22%
2-y probability of maintaining MCyR[b]	73%	74%	83%	56%	20%	31%
n[c]	63	224	26	93	30	134
Transformation to AP or BP	3%	5%	4%	4%	13%[d]	5%[d]
Deaths ≤30 days of last dose	2%	2%	12%	3%	23%	19%
2-y probability of survival[b]	87%	92%	80%	85%	43%	46%
2-y probability of PFS[b]	76%	82%	70%	76%	31%	28%

MHR, major hematologic response; CHR, complete hematologic response; MCyR, major cytogenetic response; CCyR, complete cytogenetic response.
[a]Evaluable patients had ≥1 bosutinib dose and a valid baseline assessment.
[b]Kaplan-Meier estimate. [c]Treated population.
[d]Patients with AP CML (≥65 y, n=1/8; <65 y, n=3/56) who transformed to BP.

Results. Notable baseline medical events (≥65 vs <65 years) included respiratory disorders (35% vs 13%), cardiac disorders (29% vs 9%), and diabetes (4% vs 4%). Median numbers of baseline medications were 5 (≥65 years) and 3 (<65 years). The median duration of bosutinib treatment was 11 months and the median follow-up was 31 months among both patient groups. Common on-study concomitant medications (≥65 vs <65 years) included anti-diarrheals (64% vs 57%), antacids (69% vs 41%), analgesics (60% vs 50%), systemic antibacterials (56% vs 51%), and diuretics (48% vs 20%). Bosutinib was dis-

continued by 80% of patients ≥65 years and 67% of patients <65 years; 32% and 18% of patients, respectively, discontinued due to an adverse event (AE; most commonly thrombocytopenia [6% vs 3%]), while 19% and 25% of patients discontinued due to disease progression. Rates of response were similar or numerically lower in older versus younger patients (Table). On-treatment transformation to AP or BP CML was generally similar between age groups (Table). Rates of on-treatment progression-free survival (PFS) and on-study overall survival at 2 years were numerically lower among older versus younger patients in the CP2L and CP3L cohorts, but similar in the ADV cohort (Table). Eleven (9%) older patients and 32 (7%) younger patients died within 30 days of their last bosutinib dose. Incidences of non-hematologic treatment-emergent AEs were generally similar between older and younger patients, notably (all grades/grade ≥3 for ≥65 vs <65 years): diarrhea (85%/9% vs 81%/8%), infection (56%/15% vs 49%/10%), nausea (50%/1% vs 46%/2%), vomiting (43%/3% vs 38%/3%), rash (39%/8% vs 31%/6%), fatigue (34%/3% vs 20%/2%), and edema (8%/0% vs 4%/<1%). Cardiac AEs were reported for 29% of older (atrial fibrillation [6%], congestive cardiac failure [5%], cardiac failure [5%]) and 10% of younger patients, including 15% and 4% of patients with grade ≥3 events. Of patients with cardiac events, 44% and 33% received concomitant medication, and 53% and 67% had their event resolve (median event duration, 6 vs 11 days). Dose reductions and delays, respectively, due to AEs were required by 54% and 77% of patients ≥65 years versus 41% and 60% of patients <65 years. Common grade ≥3 laboratory abnormalities (≥65 vs <65 years) included thrombocytopenia (35% vs 35%), neutropenia (21% vs 25%), anemia (19% vs 19%), hypermagnesemia (8% vs 11%), and elevated lipase (10% vs 6%). **Conclusion:** In general, bosutinib demonstrated similar efficacy and acceptable safety in older and younger patients with Philadelphia chromosome-positive leukemia and resistance/intolerance to prior imatinib and possibly dasatinib and/or nilotinib.

0758

RESULTS FROM THE ENESTND EXTENSION STUDY: EFFICACY AND SAFETY OF NILOTINIB 400 MG BID IN PATIENTS WITH PH+ CML-CP AFTER SUBOPTIMAL RESPONSE OR TREATMENT FAILURE TO IMATINIB OR NILOTINIB 300 MG BID

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In the ENESTnd study, nilotinib demonstrated superior response rates and lower rates of progression to accelerated phase/blast crisis (AP/BC) and CML-related death vs imatinib in patients with newly diagnosed Ph+ CML. Patients in ENESTnd who experienced suboptimal response or treatment failure (per European LeukemiaNet 2009 recommendations) on nilotinib 300 mg BID or imatinib could discontinue the core study and enter an extension study to be treated with nilotinib 400 mg BID; entrance was not allowed for intolerance alone. **Aims.** The purpose of the extension study was to evaluate the efficacy and safety of nilotinib 400 mg BID in patients after suboptimal response or treatment failure on imatinib (including patients on imatinib escalated to 400 mg BID) or on nilotinib 300 mg BID. Here, we report the results of the ENESTnd extension study with a median follow-up of 15-18 months. **Methods.** Patients entering the extension study were evaluated for complete cytogenetic response (CCyR) and major molecular response (MMR) on extension treatment with nilotinib 400 mg BID. Patients were also evaluated for progression to AP/BC and overall survival (OS). **Results.** Overall, 54 patients entered the extension study and received nilotinib 400 mg BID; 19 initially randomized to nilotinib 300 mg BID in ENESTnd (nilotinib dose-optimization group) and 35 initially randomized to imatinib (nilotinib second-line group). In the nilotinib dose-optimization group, 26% of patients had mutations at extension-study entry. In the nilotinib dose-optimization group, 33% of patients not in CCyR at extension-study entry and 39% not in MMR at extension-study entry achieved these responses on extension treatment with nilotinib 400 mg BID; 1 patient in the dose-optimization group progressed to AP/BC (after discontinuation from extension treatment). The estimated 18-month rate of OS was 94% in the nilotinib dose-optimization group. In the nilotinib second-line group, 69% of patients had prior imatinib dose escalation to 400 mg BID, and 31% of patients had mutations at extension-study entry. In the nilotinib second-line group, 58% of patients not in CCyR at extension-study entry and 32% of patients not in MMR at extension-study entry achieved these responses on extension treatment with nilotinib 400 mg BID. Of the 24 patients who had prior imatinib dose escalation, 15 and 23 patients, respectively, were not in CCyR and MMR at extension-study entry; 9/15 (60%) achieved CCyR and 7/23 (30%) achieved MMR on extension treatment with nilotinib 400 mg BID. Five patients in the second-line group progressed to AP/BC (2 while on extension treatment). The estimated 18-month rate of OS was 87% with second-line nilotinib. The safety profile of nilotinib 400 mg BID was consistent with previous studies, and nilotinib dose escalation appeared safe with minimal additional safety signals. **Conclusions.** These data demonstrate that nilotinib is an effective therapeutic option for patients after imatinib failure and that nilotinib dose escalation may benefit some patients with suboptimal response and treatment failure on nilotinib 300 mg BID. Additional follow-up will provide more information on this strategy.

Nilotinib Dose Optimization Group (n = 19)	
Reason for entering extension, n (%)	
Treatment failure*	3 (16)
Suboptimal response†	15 (79)
Other#	1 (5)
Response achieved during extension, n (%)	
Response prior to entry < CCyR (n = 6)	2 (33)
Response prior to entry < MMR (n = 18)	7 (39)
Nilotinib Second-Line Group (n = 35)	
Patients with imatinib dose escalation to 400 mg BID prior to extension, n (%)	24 (69)
Reason for entering extension, n (%)	
Treatment failure*	21 (60)
Suboptimal response†	12 (34)
Other‡	2 (6)
Response achieved during extension, n (%)	
Response prior to entry < CCyR (n = 26)	15 (58)
Response prior to entry < MMR (n = 34)	11 (32)

0759

DEFINITIONS AND STANDARDISATION OF 'COMPLETE' MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA

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The international, collaborative effort to standardize BCR-ABL qRT-PCR testing for CML has focused largely on determining whether a patient has or has not achieved MMR (≤0.1% BCR-ABL on the International Scale; equivalent to ≥3-log reduction in BCR-ABL transcript levels from the IRIS standard baseline). Many patients on imatinib achieve MMR, but only a minority progress to what has been termed complete molecular response (CMR), defined by the European LeukemiaNet (ELN) as undetectable BCR-ABL transcripts by qRT-PCR and/or nested PCR in 2 consecutive samples with a sensitivity >10^4. This def-

inition, however, is difficult to implement in a standardised fashion across multiple centres and does not adequately take into account variations in assay sensitivity within and between centres. Improved definition of molecular milestones is a pressing issue since second-generation TKIs result in deeper molecular responses compared to imatinib. Furthermore, there is considerable interest in conducting studies (e.g. EURO-SKI) to assess the possibility of stopping TKI therapy once significant and sustained molecular responses are achieved. There is a general consensus that it is not possible to have a single workable definition of CMR, but rather the level of response needs to be defined by an upper boundary. So, just as MMR corresponds to ≤0.1% BCR-ABL^{IS}, the terms CMR^4, CMR$^{4.5}$ and CMR^5 have started to be used to indicate levels of disease ≤0.01% BCR-ABL^{IS} (4-log reduction from IRIS baseline), ≤0.0032% BCR-ABL^{IS} (4.5-log reduction) and ≤0.001% BCR-ABL^{IS} (5-log reduction), respectively. However there are two immediate problems. The first is semantic: the fact that cut offs are defined by an upper boundary means that disease may still be detectable at a lower level, which does not fit well with the term 'complete'. We therefore propose that the terms for low level disease are modified to 'molecular response', i.e. MR^4, MR$^{4.5}$ etc. The second problem concerns laboratory standardisation: how is MR^4, MR$^{4.5}$ etc. actually determined in the testing laboratory and how comparable are results across different laboratories? ENEST1st is a phase IIIb, open-label study of nilotinib in adult patients with newly diagnosed CML (ClinicalTrials.gov NCT01061177). The primary study aim is to establish the rate of MR^4 at 18 months and to work with EUTOS (European Treatment and Outcome Study) laboratories to improve the sensitivity and standardisation of qRT-PCR for low level BCR-ABL detection. Preliminary analysis of data from the 12 ENEST1st molecular monitoring laboratories shows substantial variation in scoring of low level disease results caused principally by technical differences but exacerbated by differences in laboratory definitions. Since conversion factors are of questionable value when disease is undetectable, we suggest the following working criteria should be used to define molecular response: MR^4 = either (i) detectable disease ≤0.01% BCR-ABL^{IS} or (ii) undetectable disease in cDNA with ≥10,000 ABL or ≥24,000 GUSB transcripts MR$^{4.5}$ = either (i) detectable disease ≤0.0032% BCR-ABL^{IS} or (ii) undetectable disease in cDNA with ≥32,000 ABL or ≥77,000 GUSB transcripts . Details of these definitions, their implementation in the testing laboratory and what further work needs to be performed will be discussed.

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MULTICENTER CLINICAL STUDY EVALUATING THE CONFIRMED CMR TO MMR RATIO AMONG IMATINIB-TREATED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS USING INTERNATIONAL STANDARD RQ-PCR

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Background. Research confirming the significance of achieving a complete molecular response (CMR) during treatment of the chronic phase of chronic myeloid leukemia (CML-CP) is going to be of importance. According to the Stop Imatinib (STIM) study reported by Mahon et al., 41% of patients who achieved a CMR for at least 2 years during treatment with imatinib had sustained the CMR for at least 12 months after cessation of the drug. **Aims.** The ratio of CML-CP patients achieving CMR (BCR-ABL^{IS}≦0.0032%) among those who achieved MMR was evaluated using international standard real-time quantitative PCR (IS-PCR). In addition, the correlation between the molecular response and the clinical parameters and immunological or pharmacokinetic background. was studied. **Methods.** Patients with a confirmed diagnosis of CML-CP, who were 16 years old or older, treated with ongoing imatinib at any dose, and in MMR confirmed within 3 months before enrollment, were eligible for inclusion at 21 participating institutions in Japan. The molecular response was assessed using IS-PCR at a central laboratory (BML, Inc., Kawagoe, Japan). CMR was confirmed by analysis of blood samples collected from each patient twice on different days after getting informed consent. Flow cytometry-based analysis (FCM) of peripheral blood T-cell subsets was performed using a whole blood lysis technique. Imatinib trough concentrations were determined using high-performance liquid chromatography (HPLC) equipped with a mass spectrometric detector. *ABCG2* 421C>A (rs2231142), ABCB1 3435C>T (rs1045642), *SLC22A1* 156T>C (rs1867351), *SLC22A1* 480G>C (rs683369), and *SLC22A1* 1022C>T (rs2282143) genotyping were performed using PCR-RFLP. **Results.** Of the 157 patients registered, data from 130 were evaluated by the deadline for this abstract. Imatinib was administered for a median of 70.0 months (range: 9.7-118.5 months), and the median actual daily dose was 396 mg (range: 159-528 mg). From the results of double IS-PCR tests performed on different days, 60 patients (46%) were classified into the CMR group. Comparison of patients who did and did not achieve CMR revealed several significant differences (Table 1). First, the median time to MMR from the onset of imatinib administration was significantly shorter in patients with CMR than in those without CMR. Second, although the median trough concentration of imatinib did not significantly differ between patients with and without CMR, the frequency of *ABCG2* 421C/A or 421A/A among patients with CMR was significantly greater than among those without CMR. Third, T cell profiles in peripheral blood evaluated by FCM were related to achievement of CMR. CD4+/CD25$^{high-int}$/CD127^{low} Treg cells were significantly less frequent in patients with CMR than in those without CMR. **Conclusions**. Among CML-CP patients confirmed to have achieved MMR, about half could have achieved CMR. The correlation between the molecular response and the clinical parameters and characteristics of patients achieving a confirmed CMR are discussed.

Parameters	CMR (n=60) median (range)	no CMR (n=70) median (range)	P-value
Age, years	60.5 (28 - 85)	59.5 (22 - 81)	0.9926
Sex †, Female	19 (31.7)	23 (34.3)	0.8850
Hight, cm	164 (146 - 185)	165 (126 - 181)	0.8885
Body weight, kg	62 (42 - 83)	63 (36 - 110)	0.5373
Past history †	26 (43.3)	31 (46.3)	0.9131
Sokal risk, Low/Int/High	48 / 11 / 0	47 / 19 / 3	0.1114
EUTOS risk, Low/High	50 / 5	59 / 8	0.6118
ACA †	2 (3.3)	5 (7.1)	0.2886
CML duration, mos	79.5 (16 - 226)	67 (9 - 193)	0.2429
Prior IFN-α †	9 (15.0)	14 (20.9)	0.4564
Prior HU †	16 (26.7)	20 (29.9)	0.8088
Duration of IM treatment, mos	77.0 (16.6 - 113.0)	66.6 (9.7 118.5)	0.1577
Actual daily IM dose, mg/day	396.0 (158.8 - 527.6)	395.5 (168 486.5)	0.8747
Estimated total IM dose, g	714.0 (133.3 - 1538.0)	626.2 (61.0 1662.0)	0.1338
Good adherence †	59 (98.3)	68 (97.1)	0.5580
Median time to CCyR, mos			
From diagnosis to CCyR	7.2 (2.3 - 67.0)	6.6 (1.0 - 91.6)	0.6318
From IM therapy to CCyR	5.1 (0 - 46.6)	5.1 (0 - 51.0)	0.4555
Median time to MMR, mos			
From diagnosis to MMR	15.1 (4.4 - 185.6)	25.8 (4.4 - 167.8)	0.0575
From IM therapy to MMR	13.3 (3.2 - 76.1)	18.9 (3.7 - 101.3)	**0.0389**
IM trough concentration, ng/mL	1011.8 (760.1 - 1475.4)	1267.1 (955 - 1720)	0.1723
IM transporter SNP (n=31)			
ABCG2 *421C/A CC* vs. *ACAA* †	7 / 8	14 / 2	**0.0151**
ABCB1 *3435C/T CC* vs. *CT/TT* †	3 / 12	5 / 11	0.4544
SLC22A1 *156T/C TT* vs. *TC/CC* †	4 / 11	8 / 8	0.1826
SLC22A1 *480G/C GG/GC* vs. *CC* †	1 / 14	4 / 12	0.1655
SLC22A1 *1022C/T CC* vs. *CT/TT* †	10 / 5	8 / 8	0.3474
FCM, Median (quartile 1-quartile 3)			
CD8+ cell	247.0 (140.0 - 388.0)	288.0 (192.5 - 425.5)	0.0773
CD3-CD56+CD57+ NK cell	137.0 (5.6 - 750.4)	150.5 (26.9 - 610.3)	0.4298
CD3+CD56+CD57+ T cell	24.0 (5.5 - 54.5)	35.0 (11.5 - 61.5)	0.2833
CD4+CD25^{hi-int} CD127 ^{lo}T reg	17.5 (3.0 - 70.3)	25.0 (5.0 - 64.0)	**0.0318**

† Data presented as number (%) of patients.

0761

BONE MARROW ANGIOGENESIS AND ITS CLINICOPATHOLOGICAL CORRELATION IN CHRONIC MYELOID LEUKEMIA: A MORPHOMETRIC STUDY

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Background. Angiogenesis is classically associated with growth, dissemination and metastases of solid tumors**.** Increased microvessel density has been documented in bone marrows of various hematological malignancies including chronic myeloid leukemia (CML). **Aims.** The aim was to study multiple morphometric microvessel characteristics in CML patients and evaluate their relationship to clinicopathological parameters of proposed prognostic significance. **Methods.** Bone marrow paraffin -embedded biopsies obtained at diagnosis from 50 patients with CML in chronic phase and 10 patients in blast transformation, diagnosed at our institute during 2005-2009, were studied. Ten age and sex matched patients in which marrow biopsies were performed as part of staging procedure for non-Hodgkin's lymphoma and had no evidence of marrow disease were taken as controls. Immunohistochemically stained slides using anti CD34 antibody were examined for microvessel density (MVD) using light microscopy and computerized Image analysis system for following morphometric parameters: MVD, total vascular area (TVA), perimeter, major and minor axis length. SPSS 15 was used for statistical analysis. The data is presented as mean ±SD. ANOVA was used to compare the morphometric variables among the three groups of CML chronic phase, blast transformation and controls. **Results.** The mean MVD on image analysis was 228.7± 63.2 / mm^2 in chronic phase CML patients and 275.1± 93.8 in blast transformation patients which was significantly higher (p<0.001) than in control group 58.4±12.9. A significant difference was also observed between size related parameters i.e. TVA, perimeter, major and minor axis lengths in the chronic phase group and controls. Total vascular area in CML chronic phase 2.0±0.7% and blast phase 1.6±0.7 % was significantly higher (p<0.001) than in controls 0.3 ±0.2 %. The mean perimeter

34.4 ±7.4µm and major axis length 11.9±2.9µm in blast transformation group were lower and differed significantly from the chronic phase group perimeter 47.8±9.6 µm and major axis length 16.8±4.0µm indicating a predominance of smaller vessels with less branching in the former group. MVD correlated significantly ($p<0.01$) with TVA. A positive correlation was found between MVD and blast percentage in bone marrow of patients in blast transformation. A negative correlation existed between vessel number and vessel size parameters (though not significant). None of the morphometric variables was related to patient's age, spleen size, hemoglobin level, total leukocyte count, platelet, eosinophil or basophil counts. **Conclusion.** Our data suggests that vascularity is increased in CML in both chronic and blast phase and supports the hypothesis that changes in angiogenic parameters may participate in conversion of normal marrow to CML.

0762

THE NEW EUTOS SCORE HAS PROGNOSTIC VALUE IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) OUTSIDE CLINICAL TRIALS

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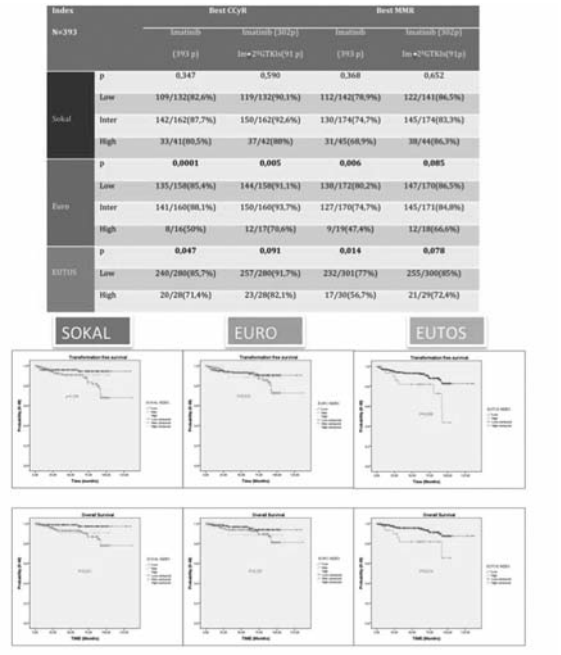
Background. Hasford et al have published a new scoring system, which was developed by the EUTOS project of the European Leukemia Net. This study of 2060 patients includes only CML patients treated with imatinib-based regimes in clinical trials.This new EUTOS score identified only two variables with independent prognostic significance, the spleen size, and percentage of basophils, variables included in the Sokal and Euro score[3], respectively. As the EUTOS score has been developed from clinical trials, there is a need of validating it outside this specific settingThe RELMC Registry is a continuous, 17-hospitals-based CML registry including patients not included in clinical trials, and whose aim is to describe the treatments received by patients with CML in Spain, their outcomes, and the variables that influence treatment choices and results.



Aims. 393 newly-diagnosed CML-CP patients have been treated upfront with imatinib, with a median age of 54,3 years, 236(60%) were men, 157 (40%) women. At diagnosis, the score distribution was as follows: Sokal L-I-H (39%-49%-12%), EURO L-I-H (47%-48%-6%); and EUTOS: L-H (91%-9%). Out of the 393 patients, 302 remained in Imatinib as sole therapy. (252 imatinib 400, 27 with high dose upfront , and 23 patients switching from Im400 to 600 or 800 mg. Ninety patients were switched to a second generation TKI (2GTKI) (20 because of intolerance, and the others 71 because of failure, suboptimal response or lost of response). Out of them, 43 received dasatinib as second line, 25 nilotinib as second line, 16 dasatinib and then nilotinib, and 7 nilotinib and then dasatinib)The responses have been analysed considering the best response obtained during the initial treatment with Imatinib, or irrespective of the scheme in which they were obtained. **Results.** Response to treatment: Our results show that EUTOS score has substantial discriminating power, as far as CCyR and MMR is concerned, specially when analysing the best response obtained with Imatinib as first scheme of therapy. Euro score discriminates only between low intermediate versus high-risk groups.Survival and progression-free survival: Out of 393 patients, 13 patients have suffered transformation (6 AP, 7 BC). In total, 25 patients have dead (6,3%).The probability of transformation was statistically significant higher in high-risk EUTOS patients, but neither Hasford nor Sokal were discriminant in this aspect. The same applies for progression-free survival and overall survival. **Conclusions.** Our results validate the EUTOS score, within the setting of TKI based therapies, away from clinical trials. In fact, EUTOS score appears more discriminant than the other prognostic systems. While waiting the definitive results of the EUTOS project to see if this scoring system has significant predictive power in larger series of patients, we feel that the EUTOS score warrants application in every single CML patient, along with the Sokal and Euro scores.

0763

LONG-TERM CLINICAL SIGNIFICANCE OF CYTOGENETIC CLONAL EVOLUTION IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB

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Background. Cytogenetic clonal evolution in chronic myeloid leukemia (CML) is considered as a step to progression to accelerated phase and blast phase (BP). However, it has been described in approximately less than 10% of chronic phase (CP) CML already at diagnosis. Long-term clinical significance of the new other chromosome abnormalities (OCA) on therapy as well as additional chromosome abnormality (ACA)/variant Philadelphia translocation (vPh) found at diagnosis in newly diagnosed CP CML patients treated with imatinib mesylate (IM) is not yet fully clarified. **Aims.** The aim of this study was to investigate long-term clinical significance of the OCA on therapy as well as ACA and vPh at diagnosis in newly diagnosed CP CML patients treated with IM. **Methods.** 281 consecutive patients newly diagnosed as CP CML at Seoul St. Mary Hospital between January 2001 and May 2009, and started IM (400 mg/day) therapy without prior treatment except hydroxyurea or anagrelide were analyzed. The median number of cytogenetic examination for each patient was 4 (range, 1-28). There were 161 males (57%) and 120 females (43%). Median age was 41 years (range, 18-77). **Results.** At newly diagnosis, 259 patients (92%) of the 281 patients had only standard Ph translocation, 13 (5%) patients had ACA(s), and 9 (3%) patients had a vPh chromosome. Of the 22 patients with CCA, 17 (77.3%) 11 (50%), and 3 (13.6%) patients have achieved CCyR, 2 consecutive MMR and $MR^{4.5}$ after a median 6.0, 12.3 and 25.7 months of IM treatment, respectively. In contrast, of the 259 patients without CCA, 227 (87.6%), 164 (63.3%), and 50 (19.3%) patients have achieved CCyR, 2 consecutive MMR, and $MR^{4.5}$ after a median 6.3, 19.8, and 24.8 months, respectively. With a median follow-up of 78.6 months (range, 1.4-126.1 months), the-5-year OS, PFS and EFS were 77.8%, 77.8% and 75%, respectively for the patients with vPh; 100%, 100% and 66.3%, respectively for the patients with ACA; 96%, 94.3% and 92.1%, respectively for the patients without CCA. The patients with vPh had the lower OS, PFS and EFS compared to the patients without CCA. Whereas the patients with ACA had no difference in OS, PFS and EFS, compared to the patients without CCA.In our study, 19 patients developed new OCA in Ph- cells (n = 16) and Ph+ cells (n = 3) on therapy. The occurrence of OCA in Ph+ cells can lead to significantly decreased OS, PFS and EFS with a RR of 20.59 ($P <0.001$), 43.32 ($P <0.001$), and 38.02 ($P <0.001$), respectively compared with the patients who had no CCA at diagnosis and no OCA on therapy (no CA group). Whereas, OCA in Ph- cells had no impact on survival outcomes compared with no CA group. **Conclusions.** This study showed that vPh at diagnosis was associated with poor survival outcomes, whereas ACA did not affect long-term survival outcomes. In addition, the distinctive difference of prognostic impact of OCA in Ph+ cells or OCA in Ph- cells on therapy may identify a group of patients requiring a change of treatment strategy.

0764

SPLICE VARIANT, ABL EXON 7 DELETION DOES NOT CONFER IMATINIB RESISTANCE IN CHRONIC MYELOID LEUKEMIA

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Background. In chronic myeloid leukemia (CML), the best characterized imatinib resistance mechanisms are BCR-ABL tyrosine kinase domain (TKD) mutations and clonal evolution, but recently alternative splicing (AS) of BCR-ABL was also proposed as a mechanism for imatinib resistance. Most of the studies focused on three splice isoforms: deletions of exons 4 or 7 (Δexon4 or Δexon7) and a 35-basepair-long insertion between exons 8 and 9. Although several groups reported the presence of Δexon7 in patients with imatinib-resistant CML on BCR-ABL (BCR-ABL$^{\Delta exon7}$) and even in healthy controls on ABL (ABL$^{\Delta exon7}$), detailed structural analysis has not been performed yet.**Aims.** The aim of this study was the characterization of Δexon7 by systematic screening of the frequency of BCR-ABL$^{\Delta exon7}$ andABL$^{\Delta exon7}$ during the disease course and in healthy individuals as well as by bioinformatic modeling. **Methods.** The frequency of Δexon7 was investigated in 30 healthy controls and in 76 CML patients by four different molecular genetic methods (Sanger-sequencing, fragment analysis, allele-specific and quantitative PCR). The functionality and viability of the variant protein was tested by bioinformatic prediction.*Results*. By sequencing, BCR-ABL$^{\Delta exon7}$ was present in 17% (12/71) of imatinib resistant patients. Higher frequency of BCR-ABL$^{\Delta exon7}$ was found in patients at the time of imatinib resistance by fragment analysis and allele-specific PCR (70% [7/10]; 100% [10/10], respectively) compared to sequencing. In serial samples from imatinib-treated CML patients, using fragment analysis and allele-specific PCR, BCR-ABL$^{\Delta exon7}$ was more frequent at diagnosis (80% [12/15] and 87% [13/15]) and at the time of resistance (70% [7/10] and 100% [10/10]), than at the time of therapeutic response (0% [0/9] and 57% [8/14]). The BCR-ABL$^{\Delta exon7}$ negative samples exhibited lower BCR-ABL expression compared to positive samples. In contrast to BCR-ABL, the frequency of ABL$^{\Delta exon7}$ was similar comparing samples collected at different time points. ABL$^{\Delta exon7}$ was detectable in 77% (23/30) of healthy control samples by fragment analysis and in 100% (30/30) by allele-specific PCR. According to secondary structure prediction by bioinformatic methods, exon 7 deleted mRNA is a target for nonsense-mediated decay (NMD) which mediates mRNA degradation. Bioinformatic analyses also suggest that a large hydrophobic surface of the protein is exposed as a result of exon 7 deletion, which triggers the unfolded protein response mechanism eliminating the truncated protein as a misfolded protein. Furthermore, ABL$^{\Delta exon7}$ protein lacks the activation loop that prevents the truncated protein to function as a tyrosine kinase. **Summary**. We concluded that Δexon7 is not associated with imatinib resistance since it is abundantly present in imatinib naive CML patients on BCR-ABL. As Δexon7 also occurs on the non-translocated (normal) ABL, the AS process is likely to be independent from BCR-ABL translocation. The detection rate of Δexon7 is highly dependent on expression levels of BCR-ABL or ABL and the sensitivity of the detection method. The low translational efficiency due to NMD degradation of the variant mRNA, together with the significant structural changes in the truncated protein and its TKD are not in favour of the possibility, that the Δexon7 isoform is associated with drug resistance in CML.

0765

PROGNOSTIC IMPACT OF ABCG2 AND HOCT1 TRANSCRIPT LEVELS IN PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB

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Background. Imatinib mesylate is a substrate for both drug efflux transporter ATP-binding cassette transporters (ABCG2; BCRP) and influx transporter human Organic Cation Transporter 1 (hOCT1; SLCA22). The clinical impact of these two transporters on imatinib-treated chronic myeloid leukemia (CML) patients remains clarified. **Aims.** The present study was performed to examine the correlation between the expression of ABCG2/hOCT1 and the clinical outcome in newly diagnosed chronic-phase CML patients treated with imatinib. **Methods.** All enrolled patients were diagnosed as chronic-phase CML and started imatinib treatment at a dose of 400 mg/day. ABCG2 and hOCT1 mRNA levels were determined by performing real-time polymerase chain reaction assays on bone marrow samples obtained at initial diagnosis from the patients. **Results.** Median age of 137 enrolled patients was 53 years (16-75 years) and median follow-up time from the start of imatinib treatment was 38 months (3-97.2 months). Of total 137 patients, 64.2%, 48.2%, and 8.0% could achieve complete cytogenetic response (CCR), major molecular response (MMR) and complete molecular response (CMR) during the whole period of imatinib treatment, respectively. The achievement of CCR or MMR during the treatment of imatinib was highly associated with progression-free survival (PFS) and overall survival (OS). Patients achieving CCR showed higher rate of 3-year PFS (92.8% vs. 46.0%, P=0.00) and OS (98.2% vs. 58.5%, P=0.00) than those without CCR. Patients achieving MMR also showed higher rate of 3-year PFS (94.6% vs. 57.6%, P=0.00) and OS (100% vs. 68.4%, P=0.00) than those without MMR. Patients with low ABCG2 expression (< 14.9 a.u.) could achieve higher rates of CCR (71.2% vs. 56.5%, P=0.04) and MMR (59.1% vs. 39.1%, P=0.02) than those with high ABCG2 expression (≥ 14.9 a.u.). The time to MMR achievement was significantly shorter in patients with low ABCG2 expression than those with high ABCG2 expression (18.1 months vs. 30.3 months, P=0.03). Patients with high hOCT1 expression (≥ 13.8 a.u.) showed significantly higher rate of intolerance with imatinib treatment than low hOCT1 expression (< 13.8 a.u.) (7.2% vs. 0%, P=0.02). There was no statistical significance in response and resistance rates to imatinib according to the hOCT1 expression levels. Although the patients with high hOCT1 expression showed a trend of shorter time to achieving CCR or MMR, there was no statistical significance. Low hOCT1 level showed the higher rate of imatinib failure (27.9% vs. 18.8%, P=0.21) than high hOCT1 expression, however, there was no statistical significance. **Conclusions.** This study revealed high expression of ABCG2 at diagnosis is related to the inferior treatment outcome in CML patients treated with imatinib. More stratified treatment plans such as the early use of newly developed tyrosine kinase inhibitors as a first line therapy may be warranted for those patients.

0766

PREGNANCY OUTCOMES AND TREATMENT REGIMENS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background. Knowledge about safe pregnancy management in chronic myeloid leukemia (CML) patients is an important issue due to the achieved high survival rates and improved life quality on tyrosine kinase inhibitors (TKI) therapy. **Aims.** To analyze the proper data of Pregnancy Registry in CML females and partners of males patients receiving TKI therapy in Russian Federation. To develop the tactics of pregnancy management in CML females in connection with cytogenetic and molecular monitoring data. **Results**. There were 33 pregnancy cases from 28 CML females on TKI therapy. 5 females had 2 subsequent pregnancies each, with different outcomes. Among 33 cases 6 artificial abortions were performed by decision of patient and physician, 1 on dasatinib, 5 on imatinib therapy. The outcomes of 27 cases when women intended to prolong pregnancy are listed below:- 19 healthy children: 16 from mothers on imatinib therapy, 2 from the same woman on 1st trimester of dasatinib therapy, 1 from woman on imatinib+hydroxyurea in 1st trimester and nilotinib from 10th week;- 3 spontaneous abortions (imatinib, 1st trimester);- 1 neonatal death (on imatinib);- 4 pregnancies ongoing now (3 on imatinib, 1 after bosutinib therapy)Only 4 of 27 females interrupted TKI uptake at conception. 23 of 27 pregnancies occurred during TKI treatment. Drug uptake was stopped from 4th-8th week, therapeutic decisions were based on the grade of existing remission.From 16 cases of childbirth on imatinib: 6 females were observed without treatment, 5 with complete molecular response (CMR) and 1 with major molecular response (MMR) at the start point. Molecular response was gradually lost during pregnancy but only one female lost complete hematologic response

(CHR). All 6 women had a good restoration of response after restarting imatinib.1 female with MMR used imatinib during the whole pregnancy period. 1 female with MMR had a cytogenetic relapse without treatment and continued on interferon alpha (IFNα).For other 8 females without MMR and different grades of cytogenetic and hematologic response a supportive treatment was used: IFNα (5 cases), IFNα plus hydroxyurea (1 case) and imatinib (2 cases). The IFNα therapy allowed to maintain CHR in 3 of 6 females; for 1 of them MMR on IFNα was achieved during pregnancy. Imatinib was used irregularly by 1 of 2 females (no proper dosage), and was effective for another one - a newly diagnosed patient who started imatinib 400 mg from 17th week of gestation.Also 14 favourable outcomes were observed in partners of CML males with a long period of TKI exposure and no treatment interruption at conception: 13 healthy infants from the CML males on imatinib, 1 child on nilotinib. **Conclusions.** Women planning the pregnancy have to achieve stable CMR or MMR, then can be observed without treatment using close molecular monitoring. The sufficient treatment regimens in case of response loss are discussable. For the known pregnancy cases of CML males partners on TKI the favourable outcomes are reported. Further data accumulation in CML Pregnancy Registry can help to develop more precise recommendations.

0767

CLINICAL AND BIOLOGICAL FEATURES OF PH+ CHRONIC MYELOID LEUKEMIA (CML) LONG SURVIVOR PATIENTS (MORE THAN 15 YEARS)

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Background. Despite the positive results achieved by Interferon (IFN) and Tyrosine Kinase Inhibitors (TKIs) in the treatment of CML, there are limited data on the follow-up of CML long-survivor patients. **Aims**. We analyze the outcome of 65 (26M,39F) patients with Ph+ CML, with a follow-up of fifteen years or longer (median follow-up 214 months; range 180-312). **Methods.** All patients were diagnosed between the years 1986 and 1997; in 19 patients (29%) follow-up was > 20 years (group A), median 272 months, and in 46 patients (71%) follow-up was between 15 and 20 years (group B), median 204 months. Sokal's risk evaluation at baseline showed that 45 (69%) patients were low risk, 12 (19%) intermediate and 8 (12%) high risk. Sixty-three patients (97%) were low risk by the EUTOS revised risk score and 2 patients (3%) were high risk. The b2a2 fusion transcript was found in 26 patients (40%), and the b3a2 transcript in 39 (60%). All patients were treated with IFN-α + Cytarabine as first line treatment for a median time of 93 months (range 8-264). Some of these patients were enrolled in the GIMEMA CML0509 trial(ASH2011-Abstract 632). **Results**. Fifty patients (77%) started Imatinib as second line therapy after a median time of 100 months from the initial diagnosis. Median time on imatinib therapy was 96 months. Thirty-six patients continued Imatinib therapy while 14 patients were switched to second line TKIs for primary resistance or loss of response (n=11) or intolerance (n=3). One patient developed a Myeloid Blast Crisis (BC) and one Lymphoid BC, after 239 months and 161 months from the diagnosis, respectively. Five of sixty-five patients were transplanted : one patient died 15 years after HSCT of disease relapse (lymphoid BC); four are alive: one (receiving Imatinib) is in Complete Molecular Response (CMR), three patients (one on Imatinib, two off therapy) are in Major Molecular Response (MMR). In our patients cohort, overall survival after a median follow-up of 214 months is 97%. Twenty-nine patients (45%) are in CMR (17 receiving imatinib, 1 dasatinib, 1 nilotinib, 4 IFN-α, 6 off therapy); 25 (39%) patients are in MMR (16 receiving imatinib, 3 dasatinib, 1 nilotinib, 2 IFN-α, 3 off therapy); 4 (6%) patients are in CCgR (2 receiving dasatinib, 1 nilotinib, 1 off therapy); 1 (1%) patient receiving imatinib is in miCyR, 4 (6%) patients receiving dasatinib are in CHR. Two patients (3%) died due to disease progression, one after 172 months (transplanted patient) and one after 272 months. In group A, 5 patients (26%) are off therapy, while in group B 8 patients (9%) are off therapy. **Conclusions**. CML management changed dramatically with TKIs but the biological role of IFN-α remains crucial; our data from this subset of long-survival patients show that 15 of them (23%) never took TKIs : 6 are in MMR after receiving IFN-α therapy and 9 are off therapy (only one transplanted) after IFN. Longer follow-up in a larger series of patients is warranted to determine peculiar biological clinical features of this subset of patients.

0768

INCREASING RELATIVE SURVIVAL IN CHRONIC MYELOID LEUKEMIA (CML): A POPULATION-BASED STUDY IN LITHUANIA

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Background. The prognosis of individual CML patients has improved with tyrosine kinase inhibitor (TKI) treatment. Imatinib became partly available in Lithuania in 2005 with complete availability in 2011. The population based outcome of CML in the TKI era is only rarely analyzed and is the subject of this report. **Aims.** To evaluate relative survival trends of CML patients in Lithuania over 2000-2009 period. **Methods.** All cases with the ICD-10 code C92.1 (CML) registered within population based Hematology Monitoring System of Lithuania (average annual population 3.422 million[1]) in 2000-2009 were identified. Incidence and mortality crude rates were represented as number of cases per 100.000 inhabitants per year. Relative survival was defined as observed survival in CML group divided by the expected survival of a comparable group from a general population. **Results.** 474 patients were diagnosed with CML in 2000-2009 (263 cases from 2000 to 2004 and 211 cases from 2005 to 2009). The median age was 64 years (range 5-94). 246 (52%) patients were male. The incidence crude rate was 1.52 in 2000-2004 and 1.25 in 2005-2009 year periods, and the mortality crude rate was 0.82 during both time periods. One- and five-year relative survival ratios (RSRs) (95% CI) increased from 0.61 (0.55 - 0.67) and 0.33 (0.27 - 0.40) to 0.81 (0.74 - 0.86) and 0.53 (0.45 - 0.62) in 2000-2004 and 2005-2009 year periods, respectively (p = 0.031, Figure 1a). RSRs were also compared between Lithuanian and Swedish[2] CML patients. One- and five-year RSRs were found to be lower among Lithuanian than Swedish patients (0.69 and 0.44 vs. 0.94 and 0.80 for patients diagnosed from 2001 to 2008). The additional calendar period of 1995-1999 was included for better characterization of overall survival (OS) trends. By Kaplan-Meier analysis, OS improved with each time period with median OS (95% CI) of 19 (14 - 28), 21 (16 - 29) and 57 (41 - 72) months in 1995-1999, 2000-2004 and 2005-2009, respectively (1995-1999 vs. 2000-2004: p = 0.096, 2000-2004 vs. 2005-2009: p < 0.001, Figure 1b).

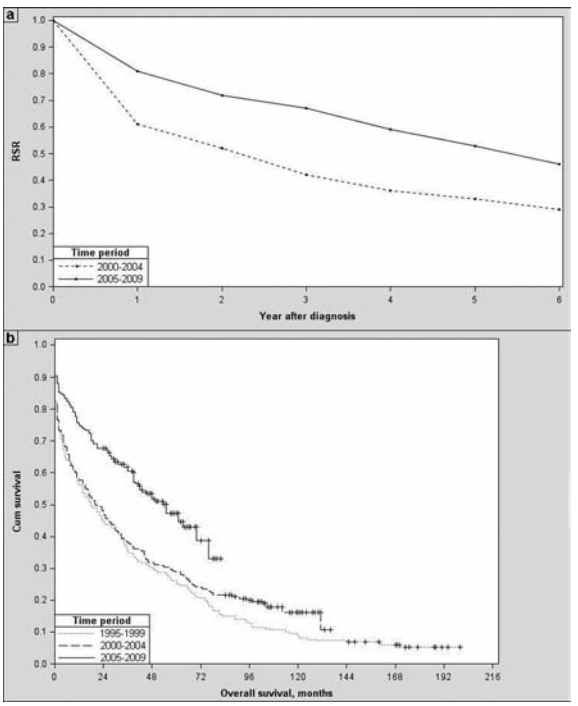

Figure 1. Relative (a) and overall survival (b) of chronic myeloid leukemia in Lithuania.

Conclusions. This population based study shows a significant improvement in outcome of CML patients in Lithuania. The most remarkable improvement in survival was observed in the last calendar period probably due to TKI treatment. However, RSRs were much lower in Lithuania as compared to Sweden which may be explained by deferred reimbursement of TKIs in Lithuania.

Reference

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0769

DETECTION OF BCR-ABL FUSION PROTEINS BY FLOW CYTOMETRIC BEAD (FC) ASSAY: PRELIMINARY RESULTS OF A PROSPECTIVE MULTICENTER SCREEN STUDY IN CHRONIC MYELOID LEUKEMIA (CML)

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Background. CML is diagnosed and monitored by cytogenetics, FISH and qRT-PCR to detect the BCR-ABL fusion gene at the chromosomal or transcript level; however, the fusion protein (FP) product of BCR-ABL is ultimately responsible for the transition to CML. Facilitating the detection of these BCR-ABL FPs may potentially complement the traditional genetic methods in CML follow-up. **Aims.** A Flow Cytometry (FC)-bead immunoassay [(BCR-ABL Protein Kit, BD Biosciences)] was evaluated to detect BCR-ABL FPs in CML in a prospective multicentre study belonging to the SCREEN group involving Hematology Centers from Sicily and Calabria, Italy. **Methods.** BCR-ABL^{POS} (p210^{+}:K562, BV173; p190^{+}:SD1) and BCR-ABL^{NEG} (HEL, HL60) cell lines, 127 peripheral blood (PB) or bone marrow (BM) samples from CML patients (n=26), ALL (n=7) and other disorders (n=5) were analyzed. PB from 32 healthy volunteers were used as controls to obtain the negative MFI cut-off values. FC-assay results were compared with those obtained by qRT-PCR normalized to BCR-ABL^{IS} and to FISH analysis. **Results.** FC-assay results using BCR-ABL^{NEG} and BCR-ABL^{POS} cell lines were concordant with qRT-PCR. Serial dilution of K562 cells in HEL cells showed the detection limit of the FC assay was >0.1%. Notably, we observed protein instability in frozen lysates and DMSO-cryopreserved patient samples with FC signals reduced by 0.05-46.9% compared to fresh samples. Time-course experiments using samples stored at 24°C (0-108h) showed comparable and progressive decline of FC signal within 48h. The presence of the BCR-ABL protein was investigated on fresh BM and/or PB from 99 samples of CML and 22 patients suspected of CML or Ph1^{+}-ALL. The FC-assay was positive in 15/15 newly diagnosed CML and in 4/4 accelerated phase CML cases (including one p230^{+}-patient). Evaluation of MRD, continued to show MFI-positive signals in 31 follow-up samples; detectable FP corresponded to 1-100% BCR-ABL^{IS}. For the remaining CML patients with undetectable FP (24), BCR-ABL^{IS} was <1%. Patients with other hematological disorders (ET, IMF, AML) tested negative both in FC and qRT-PCR. Of the Ph1^{+}-ALL cases, 2/5 pediatric patients were both FC and qRT-PCR positive, 2/2 adult ALL tested positive. We compared FISH and FC-assay; all cases testing FC-negative also tested negative for FISH, however 3/14 FC-positive cases were negative in FISH analysis although BCR-ABL^{IS} was >1%. Specifically, ROC analysis (n=89; 59 FC-positive and 30 negative) showed that a positive FC-assay could detect BCR/ABL transcripts up to BCR-ABL^{IS}=1.37% (AUC=0.97, *P*<.0001). Subdividing MFI into quartiles showed a strong proportional increase in relative %MFI corresponding to BCR-ABL^{IS} transcripts (*P*<.0001), indicating MFI values could be used as a semi-quantitative scale. **Conclusions.** The BCR-ABL FC-assay is a rapid and easy technique able to detect BCR-ABL proteins with high specificity and sensitivity. The FC-assay may thus circumvent more elaborate techniques for the initial follow-up phase of the disease, where preliminary data show that the it may be more sensitive than FISH in detecting positive samples. The SCREEN multicenter study will establish the validity of these assumptions and the relationship, if any, with cytogenetic FISH and molecular analysis using a larger cohort of CML patients.

0770

PROGNOSTIC IMPACT OF BASELINE QUALITY OF LIFE VALUES IN CML-CHRONIC PHASE PATIENTS TREATED WITH IMATINIB - 10-YEARS FOLLOW UP RESULTS

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Background. At present CML in chronic phase is not lifetime-limiting disorder. Not quantity but quality of life (QoI) becomes the major endpoint of CML management. Few efforts have been made to assess and improve QoI in CML patients. No specific intervention is included in modern CML guidelines to improve QoI. The aim of the study was to investigate prognostic significance of baseline QoI values in CML patients treated with imatinib. **Methods.** . QoI data was obtained from validation study of FACT-BRM questionnaire that was carry out in 2001-2003. Analysis was conducted on data from 93 patients with chronic phase CML treated with imatinib. Patient population consisted of 45 men and 48 women with median age 43,7 years (18,8-65,5 years) on imatinib start time. All the patients were pretreated with hydroxyurea, busulfan, interferon and various chemotherapy regimens. QoI was assessed by self-completion of Russian version of Fact-BRM at several time points: within a month before, on days 1, 8, 28, months 3, 6, 9, 12, 24 after start of imatinib. Median time between CML diagnosis was made and start of imatinib was 32 months (0-157.3 months). Stratification of patients was performed in dependence of baseline QoI values.Statistical methods included cluster analyses by Ward's method, non-parametric Kruskall-Wallis ANOVA and Median test, repeated measures ANOVA in Statistica 7.0. Missing items in questionnaires were processed with mean substitution. **Results.** The numbers of completed QoI questionnaires was declined in time - 93, 89, 86, 82, 82, 78, 78, 76 and 62 patients according to study points. All the patients were divided on two groups (38 and 55 patients) with similar linkage distances according to cluster analysis of baseline QoI values. These groups were not statistically different according age and gender of patients. QoI profiles of all datasets were very similar to IRIS results. QoI changes according to strata groups demonstrated statistically significant differences from baseline to final point in all scales with exception of Social/family wellbeing scale. The QoI parameters in group 2 was stable over time whereas in group 1 QoI had gradually increased. The composition scale - TOI profiles presented in fig.1. Results of treatment in strata groups did not reach statistical significance, but there were strong tendencies in respect to Overall Survival (p=0.19) and Event-free Survival (p=0.27). Overall 10-years survival in all patients was 80.6% with difference between groups: 86.8% (group 1) and 76.3% (group 2). A significant (p=0.047) difference was revealed concerning the probability of switching to second-generation-TKI: 42.1% (group 1) and 21.8% (group 2). These findings could be influenced by small population of patients in both groups and censored data. **Conclusions.** QoI is important characteristic of CML patients. Existing standards of care did not pay any attention to QoI measurement and management. Results of our QoI monitoring are similar to those obtained from other studies. Baseline QoI values are determine profile of subsequent QoI and can influence patients' outcome. There is a need for more active incorporation of QoI assessment into future clinical trials and routine practice.

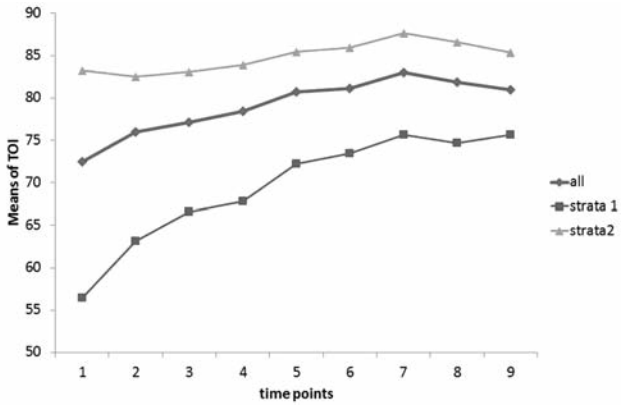

Figure 1. FACT-TOI means profile over time in stratification groups.

771

QUALITY OF LIFE AND SYMPTOM PROFILE IN PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT CHRONIC MYELOID LEUKEMIA

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Introduction of imatinib into chronic myeloid leukemia (CML) therapy marked a major advance in CML treatment. Despite remarkable responses with imatinib in chronic phase CML (CML-CP), there remains a proportion of patients who are resistant or intolerant to imatinib treatment. Importantly, without effective therapeutic intervention, these patients inevitably progress to advanced phases of disease, have a short survival and declined health outcomes. This emphasizes the importance of patient-reported outcomes in this patient population. Information about quality of life (QoL) and symptom profile in imatinib-resistant or -intolerant CML-CP patients is lacking**.** We aimed to study QoL parameters and symptom profile in imatinib-resistant or -intolerant CML-CP patients.33 CML-CP patients resistant or -intolerant to imatinib before second-line therapy were enrolled in the study (mean age - 47 years old, range - 22-71 years; male/female - 15/18). The median of disease duration was 6 years (2-13 years). Half of patients received imatinib 600 or 800 mg daily; the median duration of imatinib treatment - 42 months (4-101 months). 30 patients had resistance to imatinib treatment; 3 patients were intolerant to imatinib. The patients filled in the SF-36 for QoL assessment and Comprehensive Symptom Profile in Chronic Myeloid Leukemia Patients (CSP Leuk-CML) for symptom assessment. The CSP Leuk-CML is developed to assess profile of 47 symptoms specific for patients with CML. To compare patient population with normative data the sample from population norm (PN) data base adjusted to age and gender was used. For comparisons Mann-Whitney test was used. Symptom severity and percentages of patients with symptoms at moderate-to-severe (ratings ³ 5) levels was evaluated.Imatinib-resistant or -intolerant CML-CP patients experienced impaired QoL as compared to population norms: the values for the majority of SF-36 scales were significantly lower than in control group ($p<0.05$). No QoL impairment was observed in 32% of patients. 26% of patients had either mild (Integral QoL index <25% decrease from a PN) or moderate (25-50% decrease from a PN) QoL impairment; other 26% of patients - severe QoL impairment (50-75% decrease from a PN, and 16% of patients - critical QoL impairment (>75% decrease from a PN).The majority of patients (96%) experienced fatigue; a half of them suffered from moderate-to-severe fatigue. 75% of patients experienced at least one moderate-to-severe symptom; 13 patients (41%) had more than 7 moderate-to-severe symptoms. Almost half of patients experienced moderate-to-severe excessive sweat at rest and during mild physical activity; about one third of patients experienced moderate-to-severe fatigue (n=12), edema (n=12) and decreased work energy (n=10). Four patients experienced more than 20 (22-33) moderate-to-severe symptoms and had critical QoL impairment.Thus, QoL in imatinib-resistant or -intolerant CML-CP patients is significantly deteriorated. Fatigue is the leading symptom in this patient population. The majority of patients experience moderate-to-severe symptoms. Information about QoL and symptom profile in this patient population should be used should be used for comprehensive assessment of risks and benefits of second-line treatment of CML-CP.

0772

COST-EFFECTIVENESS ANALYSIS OF NILOTINIB VERSES IMATINIB FOR THE TREATMENT OF CHRONIC PHASE PHILADELPHIA CHROMOSOME POSITIVE CHRONIC MYELOID LEUKAEMIA

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Background. Nilotinib is a tyrosine kinase inhibitor (TKI) for the treatment of adult patients with newly diagnosed Philadelphia chromosome positive (Ph+) chronic myeloid leukaemia (CML) in chronic phase. The ENESTnd phase III trial demonstrated that nilotinib has clinical superiority over current standard treatment of first-line imatinib in patients with chronic phase Ph+ CML, on the basis that fewer patients progressed to accelerated phase/blast crisis. Whilst the clinical benefits of nilotinib have been demonstrated, the cost-effectiveness of first-line nilotinib has not been explored. **Aims**. To evaluate the cost-effectiveness of first-line nilotinib followed by second-line dasatinib compared to first-line imatinib followed by second-line dasatinib for patients newly diagnosed with chronic phase Ph+ CML. **Methods.** A Markov state-transition model based on the 24 month follow-up from the ENESTnd randomised controlled trial was developed to simulate the transitions of a hypothetical cohort of patients over a lifetime. The model estimates when one treatment will fail and hence the patient is switched to an alternative treatment. Patients who discontinue first-line treatment go on to second-line dasatinib. Patients who fail on second-line dasatinib receive stem cell transplantation or HU therapy. Patients transition from chronic phase, to accelerated phase, to blast crisis, to CML-related mortality. Patients may die from other causes at any time. EQ-5D utilities were applied to patients in each health state and utility decrements were estimated for patients experiencing severe (grade 3 and 4) adverse events (AEs) on TKI therapy. Costs (2010/11 Sterling) were estimated from the perspective of the UK National Health Service (NHS) and Personal Social Services (PSS). These include the costs associated with the different drug therapies, stem cell transplantation, routine hospital appointments for administration and monitoring, and treatment for severe AEs. A patient access scheme is available for first-line nilotinib therapy and is included in the analysis. All costs and QALYs were discounted by 3.5% as recommended by NICE. Probabilistic sensitivity analysis (PSA) was conducted to explore the impact of the joint uncertainty of all model parameters on the cost-effectiveness results. Cost-effectiveness was expressed in terms of incremental cost per quality-adjusted life-years (QALYs) gained. Cost-effectiveness acceptability curves (CEACs) were also generated. **Results**. The mean undiscounted survival in the nilotinib arm was estimated to be 13.96 years compared to 13.32 years in the imatinib arm. Over a lifetime horizon patients are estimated to gain an additional 0.64 life-years (LYs) and 0.49 quality-adjusted life-years (QALYs) in the nilotinib arm compared to the imatinib arm. Using a discount rate of 3.5% patients are estimated to accrue an additional 0.35 LYs and 0.28 QALYs in the nilotinib arm compared to the imatinib arm. Expected lifetime (discounted) costs in the nilotinib arm were £220,416 compared to £232,941 in the imatinib arm. The nilotinib arm dominates the imatinib arm as it is more effective and less costly. **Conclusions**. The results suggest that nilotinib produces improvements in survival and QALYs compared to standard treatment of first-line imatinib and it is likely to offer a cost-effective use of NHS resources.

Aggressive lymphomas 2

0773

SIL INDEX AS A NEW PROGNOSTIC PREDICTOR IN DIFFUSE LARGE B-CELL LYMPHOMA

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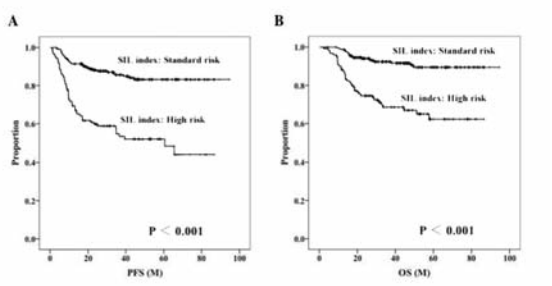
Background. Rituximab (R) plus CHOP chemotherapy (R-CHOP) is the widely standard treatment for diffuse large B-cell lymphoma (DLBCL). The revised International Prognostic Index (R-IPI) was established in 2007 as a prognostic indicator for R-CHOP therapy. **Aims.** To reassess the utility of R-IPI and evaluate soluble interleukin-2 receptor (sIL-2R) level as a prognostic factor.
Methods. The Yokohama City University Hematology Group in Japan has uniformly and curatively treated DLBCL patients since 2003. Between 2003 and 2009, 554 consecutive patients with DLBCL were registered and treated. All DLBCL patients scheduled to undergo primary therapy with 6 cycles of full-dose R-CHOP were included in this study. From among them, patients over 70 years old with an Eastern Cooperative Oncology Group performance status greater than 1 who were treated with a reduced dose were excluded; however, according to the decision of the attending physician, if they were treated with full-dose R-CHOP, they were included. Patients for whom the initial therapy dose was reduced by □20% due to any major comorbidity were excluded. Those with human immunodeficiency virus infection were also excluded. Finally, 366 of the initial 554 patients were retrospectively analyzed. Patients who had partial remission (PR) after the 4 initial cycles were administered 8 R-CHOP cycles in total, while patients who did not achieve PR after the 4 initial R-CHOP cycles or those who exhibited disease progression at any given time received salvage therapy. Additional local irradiation was also performed in patients with PR or complete remission (CR), if deemed necessary by the attending physician. No patient received maintenance therapy with R. Patients with DLBCL who achieved CR but were initially at risk of central nervous system (CNS) involvement also received intrathecal CNS prophylaxis



Results The median age at diagnosis was 64 years (range, 18 -80 years). The median number of therapy cycles was 6 (range, 2 -8), and 91% of the patients received 6 cycles or more. Forty patients (11%) received radiation therapy as primary treatment, usually to treat sites of residual masses at the end of the chemotherapy. CNS prophylaxis was conducted in 45 patients (12%). Progression-free survival (PFS) curves in "very good"and "good"risk groups, as defined by the R-IPI showed no statistical difference. We added sIL-2R level to the factors comprising the R-IPI. Five levels of sIL-2R (1000, 1500, 2000, 2500, and 3000 U/ml) were weighed with respect to their impact on PFS, and an sIL-2R level of □2,500 U/mL was determined as the most appropriate threshold. We developed a new prognostic indicator, SIL index, which involves 3 independent prognostic risk factors: clinical stage (S), sIL-2R level over 2,500 U/mL (I), and elevated LDH level (L). This index indicates standard risk (0 or 1 risk factor: 5-year PFS, 83%; 5-year overall survival (OS), 89%) and high- risk (2 or 3 risk factors: 5-year PFS, 52%; 5-year OS, 62%) outcomes (Figure 1). **Conclusions** The SIL index is a simple and objective prognostic index for DLBCL patients to identify candidates for experimental therapy other than R-CHOP.

0774

CAUSES OF DEATH AFTER COMPLETE REMISSION OF AIDS-RELATED NON-HODGKIN'S LYMPHOMA

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Background. The use of highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of AIDS-related Non-Hodgkin lymphoma (NHL), causing a remarkable increase in patients' long-term survival. With the introduction of HAART, the longer survival of HIV-patients after lymphoma treatment put them at risk of death due to causes different from lymphoma progression, such as second neoplasms and infections. There is scarce information regarding causes of death in HIV-infected patients who have responded to NHL treatment. **Aims.** We sought for causes of death among patients treated of AIDS-related NHL who achieve complete remission (CR), comparing those treated with HAART with those who did not receive it. **Methods.** We conducted a retrospective study of patient data on AIDS-related NHL individuals diagnosed between 1989 and2010 in our institution. Demographic, HIV infection, and lymphoma data on each case were collected. Two groups were considered: patients who did not take HAART at all, and those who started HAART at any stage of their HIV infection. Continuous and categorical variables are presented using descriptive statistics. Survival analyses were performed using the Kaplan-Meier method, and compared using the log-rank test. P-valuesof lessthan 0.05 were considered statistically significant.

Table 1a. Causes of death of patients after CR of NHL.

	Lymphoma progression	Second neoplasm	Infection	Other/Unknown
No-HAART	5 (50%)	0 (0%)	4 (40%)	1 (10%)
HAART	1 (8%)	4 (33%)	4 (33%)	3 (25%)

Table 1b. Comparison of CD4 lymphocyte counts and HIV load in patients from the HAART group who died versus those alive.

	Dead	Alive	P
Undetectable HIV load	4/9	22/25	0.020
median CD4 lymphocyte count/µl (range)	108 (9-199)	398 (82-1064)	<0.001

Results. Out of a series of 146 patients diagnosed with AIDS-related NHL, 139 patients were eligible for the study with a median (range) follow-up of 8.24 years (0.68-15.78): 70 patients belonged to the no-HAART group, and 69 to the HAART group. Patients in the HAART group were older at the time of lymphoma diagnosis than in the no-HAART group with a median (range) of 40 (26-68) and 34.5 years (19-66), respectively (p=0.002). There were more female patients in the HAART group: 29% versus 11% (p=0.01). A higher number of CR was observed in the HAART group: 62% versus 25%, (p<0.001). The 5-year overall survival and disease free survival were significantly longer for patients receiving HAART: 49% (95%CI: 37-61%) versus 3% (95%CI: 0-7%); and 92% (95%CI: 83-100%) versus 17% (95% CI: 0-46%), respectively. The causes of death were significantly different comparing both groups (p=0.049) (Table 1a). Four patients died of second malignancy in the HAART group (hepatocarcinoma, anal carcinoma, carcinoma of vagina and Kaposi's sarcoma), and none in the no-HAART one. However, similar percentages of deaths of infection were seen in both groups. In the HAART group, patients who died had lower CD4 counts and higher HIV loads than those patients kept alive (table 1b). Median time from CR to death of lymphoma progression was14.20 months (7-25.90) and to death of second neoplasm 112.27 (11.17-149.17). Patients who died from infection had similar median time from CR to death in both groups:9.23 months (1.47-57.77) in the non-HAART, and 6.85 (4-17.07) in the HAART. **Conclusions**. Causes of death among HIV-patients on HAART after CR of NHL are second neoplasms and infections rather than lymphoma progression. HIV-infected patients on HAART who die after treatment of NHL have poor control of HIV infection.Grants: RD06/0020/1056 from RTICC and EC11-041 from Ministerio de Sanidad.

0775

FACTORS ASSOCIATED WITH FEBRILE NEUTROPENIA IN A PROSPECTIVE COHORT OF DIFFUSE LARGE B CELL LYMPHOMA

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Background. Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma, and rituximab-CHOP (RCHOP) is currently the standard chemotherapy in patients DLBCL. Chemotherapy-induced neutropenia and its complications are one of the major concerns in cancer chemotherapy. Especially, lymphoma belongs to the type of cancers which designated as risk factors for febrile neutropenia (FN), and R-CHOP is one of the common chemotherapy regimens associated with FN with a 19% of FN risk. However, it has been rarely studied in a homogenous group with regard to their pathologic diagnosis of DLBCL and received the current gold standard R-CHOP regimen. **Aims.** In this study, we attempt to evaluate the factors associated with FN in our prospective cohort with DLBCL who were treated with R-CHOP regimen. **Methods.** From September 2008 to June 2011, a total of 829 lymphoma patients were registered in the prospective cohort of Samsung Medical Center (Samsung Medical Center Lymphoma Cohort Study, SMCLCS, NCT#00822731). At diagnosis, their comprehensive baseline characteristics including disease-related factors and host-related factors were evaluated. Furthermore, data regarding treatment for lymphoma, treatment response and survival were regularly updated by primary physician and physician assistant nurse. From these prospective cohorts registered, 378 patients diagnosed with DLBCL were exclusively selected. Prophylactic G-CSF was not routinely administered because it was not reimbursed by insurance in Korea. **Results.** After excluding patients with CNS DLBCL, and patients who received anti-cancer treatment more than R-CHOP as first line treatment, 262 DLBCL patients who had completed R-CHOP cycles as first line treatment were analyzed in this study. The median age was 55 years (range, 16-86), and 116 patients (44.3%) presented with advanced stage disease. The distribution of patients according to IPI was 131 (50%, low), 47 (17.9%, low intermediate), 41 (15.6%, high intermediate) and 43 (16.4%, high), and the median number of R-CHOP cycle was 6. Of the total 262 patients, 118 patients (45%) had ever experienced FN during R-CHOP treatment. The initial episode of FN was more commonly observed in earlier courses of treatment with more than half (60.1%) of hospital visit for FN occurred during the first two chemotherapy cycles. To determine the risk factors associated with development of FN, both univariate and multivariate analyses were performed. In univariate analysis, there was no difference in the development of FN based on extralymphonodal involve, B symptoms, sex, nutritional status measured by skin fold or MAMC, smoking and/or alcoholic behavior, whereas stage, BM involvement, IPI score, LDH, Hb, age (≥60), prealbumin, transferrin level, and ECOG performance status showed significance for the development of FN. In multivariate analysis using clinically significant variables identified by univariate analysis, however, age (≥60) was the only significant factor which affects development of FN. **Conclusions**. Among the various clinical variables, old age (≥60) was the most significant risk factor associated with development of FN in R-CHOP treated DLBCL patients.

0776

EVIDENCE FOR RITUXIMAB UNDERDOSING IN SUBPOPULATIONS OF ELDERLY PATIENTS WITH DLBC: RESULTS OF THE RICOVER-60 STUDY OF THE DSHNHL

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Background. Gender and weight independently influence rituximab clearance and serum elimination half-life. **Aims.** Therefore, we investigated the impact of rituximab pharmacokinetics on outcome of elderly DLBCL patients. **Methods.** The outcome of subpopulations of the 1222 patients treated in the RICOVER-60 trial with shorter rituximab half-lives was analyzed: elderly male vs. female patients and elderly weighty (upper quartile: >77kg) vs. slim (lower quartile: ≤60 kg) female patients. **Results.** The calculated rituximab half-life was 24.7 days in males and 33.4 days in females. Male gender was associated with a slightly increased risk for progression (relative risk: EFS: 1.0; PFS: 1.27; OS 1.3; p>0.05) after CHOP-14 without rituximab, but evolved as a significant risk factor when rituximab was added (relative risk EFS: 1.4 [p=0.016]: PFS: 1.692 [p=0.004], OS: 1.6 [p=0.063]). Addition of rituximab resulted in a significantly improved 3-year PFS (74% vs. 49%; p=0.002) in female patients with a body weight within the lower quartile (rituximab half-life: 41.1 days), while there was no improvement by the addition of rituximab (71% vs. 72%; p=0.816) in female patients with a body weight in the upper quartile (rituximab half-life: 26.1 days). **Conclusions.** The reduced benefit of adding rituximab to CHOP in elderly DLBCL patients with a shorter rituximab half-life (and consequently lower rituximab serum levels) strongly suggests that the respective subpopulations (males and weighty females) are underdosed when the rituximab dose is based on body surface area at 375 mg/m^2. Novel CD20 antibodies which are usually higher dosed should be compared with an optimized rather than the currently used suboptimal dose and/or schedule of rituximab. *Supported by deutsche Krebshilfe.*

0777

FIRST-LINE TREATMENT OF DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) DRIVEN BY EARLY PETSCAN: EVALUATION AT 5 YEARS OF A SINGLE CENTER PROSPECTIVE STUDY

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Background. In 2006, the R-CHOP regimen (Rituximab, Adriamycine, Cyclophosphamide, Vincristine and Prednisolone) was considered as the standard first-line treatment of DLBCL for fit patients. Fourteen days interval between two cycles, with a total of 6 cycles (+ 2 rituximab) was considered at that time the best approach according to Ricover and Mint's trials (respectively for elderly patients and good prognosis young patients). Subsequently, interim Petscan , ABC/GCB type and R-IPI appeared of interest as new prognostic factors. **Aims.** Validate the most recent literature data concerning the first-line treatment for fit DLBCL patients in a prospective cohort of patients from a single institutionMethods:From July 2005 to January 2011, new DLBCL patients, with negative Petscan after 3cycles were treated with 6 R-CHOP14 combined with G-CSF(D6-12). If the 14 days intervals could not be applied for any reasons, or if early Petscan remained positive, eight courses were distributed. Exclusion criteria were: transformed indolent lymphoma, age > 80 years, PS-ECOG >3, left ventricular systolic function < 50% or severe renal or hepatic dysfunction unrelated to lymphoma. Responses were evaluated using Cheson criteria (1999). Early Petscan after 3 courses and at the end of the treatment was evaluated by the visual method. Hans's algorithm was applied to determine the ABC/GCB types and all patients were classified with the R-IPI score.Results60 patients were included, 57 were centroblastic, 40% (24/57) GCB and 60% ABC. There were 41(68%) patients > 60 years old, 40 (67%) with AAstage III-IV, 11 (18%) with a PS>1, 10 (17%) with more than one extranodal site and 19 (32%) with elevated LDH level. Hence R-IPI was 0 for 9 patients (15%), 1-2 for 27 (45%) and 3-5 for 24 (40%). After a median follow up of 29.5 months, 13 died (10 for lymphoma, 1 for digestive hemorraghe and 2 for relapse of previous other cancer). At the end of treatment 51 (85%) were in CR. Fifty two patients (91 %, 52/57) had an early negative Petscan, and 5 (9%) a positive Petscan.Three years OS is 84% and is statistically affected by R-IPI [0-2 = 100% vs 3-5=60% (p<.0001)] and early Petscan [negative=95% vs positive=30% (p<.0001)] but not ABC/GCB (p=0.6). Moreover the 3 years OS of R-IPI 3-5 and negative early Petscan population is only 65%.Three years PFS is 70% and was affected by R-IPI [0-2 = 96% vs 3-5=30% (p<.0001)] and early Petscan [negative=77% vs positive=53% (p<.006)] but not by ABC/GCB status (p=.54). Moreover the 3-years PFS for negative Petscan in R-IPI 3-5 population is only 40%.ConclusionWe confirm the good results of 6 R-CHOP14 for newly diagnosed DLBCL especially for R-IPI 0-2 having a negative early Petscan despite 6 Rituximab instead of 8. (asking the question of the necessity of 8 rituximab injections for those patients). The OS of R-IPI 3-5 remains very poor regardless ABC/GCB subtypes even for early negative Petscan. New approaches will be appropriate to overcome this very bad prognosis group.

0778

HIGH DOSE ETOPOSIDE AS AN EFFECTIVE PBSC MOBILIZATION REGIMEN IN PATIENTS WITH NHL PREVIOUSLY TREATED WITH RITUXIMAB-CHOP OR CHOP CHEMOTHERAPY

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Background. Etoposide is one of the effective mobilizing agents in non-Hodgkin's lymphoma (NHL), but efficacy and toxicity of high dose etoposide followed by G-CSF compared with other mobilization regimens are not well defined. **Aims.** We conducted a retrospective study to evaluate the efficacy of high dose etoposide plus G-CSF compared with other mobilization regimens in peripheral blood stem cell (PBSC) mobilization. **Methods.** A total of 51 patients with NHL who were treated only with Rituximab -CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) or CHOP chemotherapy and sequentially underwent PBSC mobilization between 2006 and 2011 were analyzed. Twenty nine patients received etoposide 500mg/m² at day 1,2,3 (VP16

group). Total 22 of other patients received ICE (ifosfamide, carboplatin, etoposide) or DHAP (cisplatin, cytarabine, dexamethasone) or R-CHOP and 2 patients received G-CSF only (others group). All patients administered G-CSF 10μ/kg/day until apheresis completed. Efficacy of PBSC mobilization and chemotherapy related toxicities were compared between the groups.

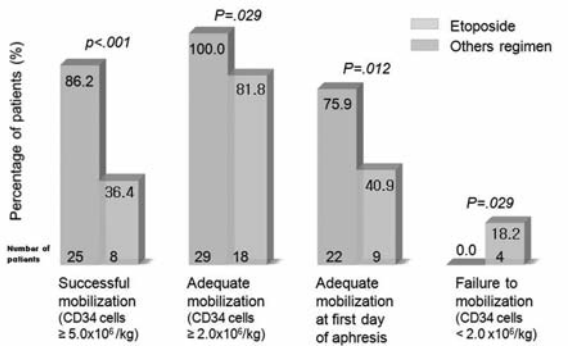

Figure 1. The results of PBSC mobilization in high-dose etoposide regimen group (left bar) and others regimen group (right bar).

Results. Both groups were comparable in age, gender, diagnosis, stage, the presence of bone marrow involvement at diagnosis, the number of previous chemotherapy, a disease status prior to mobilization and the number of days from last chemotherapy to start of mobilization chemotherapy. In the VP16 group, a median total $CD34^+$cells collected was 16.22 x 10^6 cells/kg, 75.9% of all patients having adequate (>2.0 x 10^6 cells/kg) $CD34^+$ collections after first day of apheresis compared with a median in the others group of 4.1 x 10^6 cells/kg (P<0.001), with 40.9% having adequate collection after first day (P=0.012) (Figure 1). A median total $CD34^+$cells collected per aphresis was 16.22 x 10^6 cells/kg in VP16 group and 1.97 x 10^6 cells/kg in others group (P<0.001). None of 29 patients in VP16 group and 4 of 22 (19%) in others group failed to collect sufficient $CD34^+$ cells (<2.0 x 10^6 cells/kg) (P=0.029) (Figure 1). In multivariate analysis, successful stem cell mobilization was independently influenced by high-dose etoposide regimen (P=0.044) and $CD34^+$ cells in peripheral blood at the day of apheresis (≥ 20x10^6/L) (P=0.002). Neutropenic fever developed in 20 patients (69%) in the VP16 groups, none of which were fatal, and 3 patients (14%) in the others group (P=0.003). **Conclusions**. High dose etoposide improves the effectiveness of mobilization with more than threefolds higher stem cell yield compared with other mobilization regimens. Considering no mortalities and grade IV infections in patients with neutropenic fever, high dose etoposide plus G-CSF is a highly effective mobilization regimen with acceptable toxicity in patients with NHL.

0779

RITUXIMAB COMBINATION WITH CHOP MAY NOT OVERCOME THE POOR PROGNOSIS IN PATIENTS WITH EPSTEIN-BARR VIRUS POSITIVE DIFFUSE LARGE B CELL LYMPHOMA OF THE ELDERLY

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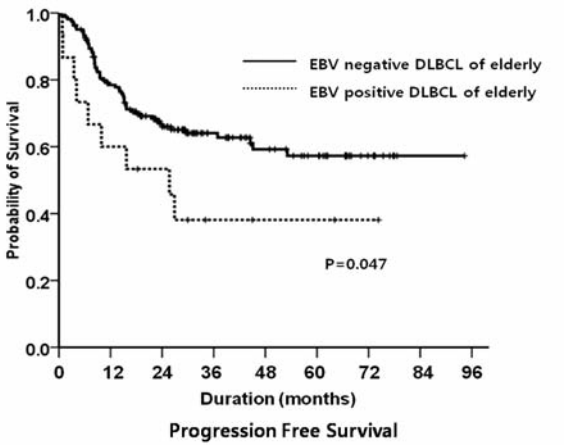
Background. Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) of the elderly was included as a provisional entity in the 2008 WHO lymphoma classification. In patients with DLBCL, the incidence of EBV among patients of Asian ranges from 9% to 15%. Few published studies support a possible prognostic relationship between EBV tumoral status and DLBCL especially in rituximab era. **Purpose**. To evaluate the prognostic value of the EBV positive DLBCL of elderly in patients with treated by rituximab, cyclophosphamide, adriamycin, vincristine and presnisone (R-CHOP). **Patients and Methods.** Among the 228 patients included on the trial, histological material was available for a total of 179 patients at diagnosis. All 179 tissues were available for analysis by EBV-encoded RNA-1 (EBER) in situ hybridization. **Results.** Sixteen cases (8.9%) were identified as EBER-positive. Interestingly, all patients with EBV positive DLBCL of elderly was diagnosed as non-germinal center B (GCB)-like DLBCL that was based on the algorithm by Hans. The EBV negative DLBCL group showed 68.8% of non-GCB like DLBCL (p=0.009). There was no clinical difference between EBV positive and EBV negative DLBCL in terms of age, Ann Arbor stage, LDH level, B symptom, number of extranodal involvement and Eastern Cooperative Oncology Group performance status at diagnosis (p>0.05). After median 6th R-CHOP chemotherapy, the clinical response rate (≥PR) was 75% (12/16) in EBV positive and 89% (145/163) in EBV negative DLBCL of elderly (p=0.27). At a median follow-up of 19.2 months, EBV positive DLBCL of elderly demonstrated the poorer median progression free survival (EBV+ vs EBV- 25.7 months [95% confidence interval 5.8-45.6 months] vs not reached, p=0.047), but it could not showed the statistical difference in overall survival (p=0.292). We could not find the difference of progression free or overall survival between GCB or non-GCB like DLBCL. **Conclusions**. R-CHOP chemotherapy may not overcome the adverse prognostic influence of EBV-positive DLBCL of the elderly and this needs confirmation by long term follow-up and prospective study.



0780

SAFETY, PHARMACOKINETIC/PHARMACODYNAMIC PROFILES AND EFFICACY OF SAIT101, A BIOSIMILAR OF RITUXIMAB IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Rituximab (Mabthera®) is a chimeric anti-CD20 monoclonal antibody indicated for the treatment of non-Hodgkin's lymphoma (NHL) and rheumatoid arthritis. SAIT101 is a biosimilar to rituximab. **Aims.** The purpose of this study is to assess safety, pharmacokinetic/pharmacodynamic (PK/PD) profiles and preliminary efficacy after intravenous infusion of Mabthera® and SAIT101 in patients with diffuse large B-cell lymphoma (DLBCL). **Methods.** This is a randomized, multi-center, open-label, parallel group study in 24 Korean patients with histologically proven DLBCL randomly assigned to receive CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) chemotherapy plus either SAIT101 or Mabthera® (375mg/m2) up to cycle 2. Then all patients received Mabthera® and CHOP up to total cycle 6 to 8. The primary objectives were to compare safety and PK and secondary objectives were to compare PK and primary efficacy of SAIT101 and MabThera®. For PK analysis, blood samples were collected at 0 (pre-dose), 2, 6 (end of infusion), 7, 8, 12 (Day 1) and 24 hours (Day 2), and on Days 4, 8, 11, 15, and 22 (before the cycle 2 infusion) after first dosing. The data was analyzed by a non-compartment method using WinNonlin. For PD analysis, change from baseline CD19 positive B-cell count (%) in peripheral blood was calculated up to 72 hours post-infusion in first cycle. For efficacy analysis, tumour response evaluation was done based on revised IWG criteria after completion of cycle 2 treat-

ment. **Results.** At the time of interim analysis, 24 patients were enrolled and no subject has withdrawn from the study. PK parameters were determined in 22 patients. The geometric least square (LS) means of area under the concentration-time curve (AUClast) and maximum concentration (Cmax) in first cycle were 26377 and 28657 hr*µg/ml and 201 and 215 µg/ml for SAIT101 and MabThera®, respectively (Figure 1). The ratio of geometric LS means (90% confidence interval, CI) of SAIT101 versus Mabthera® were 0.92 (0.78~1.09) for AUClast and 0.93 (0.78~1.13) for Cmax(Table 1). The LS means of change from baseline (72hr - 0hr) of B-cell(%) in SAIT101 and MabThera® group were -7.7 and -8.0, respectively and the difference between two groups was 0.3% (90% CI, -0.9 - 1.4). Tumor response assessment was performed in 13 patients (n = 6 for SAIT101, 7 for Mabthera®) at the time of interim analysis. The number of patients showing complete or partial responses was 2 and 4 in SAIT101 group and 3 and 4 in Mabthera® group. Safety population included all 24 patients who received at least one cycle of treatment with SAIT101 or of SAIT101 and MabThera® and CHOP. A total of 39 SAEs from 12 patients were reported. Most frequently reported adverse events were neutropenia, fatigue, nausea, dyspepsia, and decreased appetite and there was no safety signal of difference between two groups. There was one mortality case with drug-induced pneumonitis in SAIT101 group and no suspected unexpected serious adverse reaction (SUSAR) case. **Conclusions.** The study results show high probability of PK and PD equivalence of SAIT101 to MabThera® and preliminary safety and efficacy profiles of SAIT101 comparable to those of MabThera®.

Figure 1. Mean concentration-time curve in each group

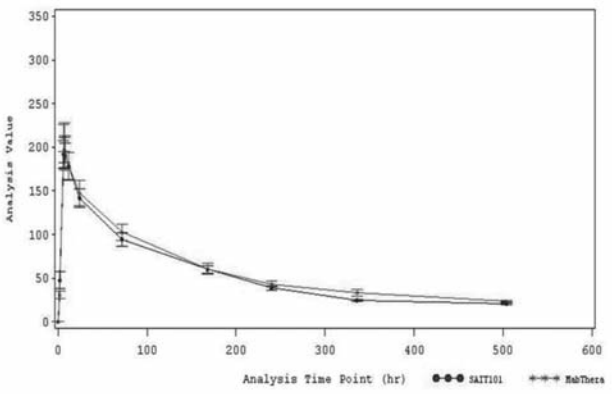

Table 2. Comparability of primary PK endpoints – AUC_{last} and C_{max}

	SAIT101 (n = 11)	MabThera® (n = 11)
AUC_{last} (hr*µg/ml)		
Geometric LS mean	26377.39	28656.55
Geometric LS mean ratio (SAIT101/MabThera®)	0.92	
90% CI for geometric LS mean ratio	[0.778, 1.089]	
C_{max} (µg/ml)		
Geometric LS mean	201.4	215.4
Geometric LS mean ratio (SAIT101/MabThera®)	0.93	
90% CI for geometric LS mean ratio	[0.777, 1.125]	

0781

TREATMENT INTENSIFICATION FOR HIV-POSITIVE PATIENTS WITH HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA DOES NOT IMPROVE OUTCOME: A RETROSPECTIVE, MULTI-CENTRE STUDY

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Background. Since the introduction of combination antiretroviral therapy (cART), the outcome for patients with diffuse large B-cell lymphoma (DLBCL) and HIV infection is similar to that of HIV-negative patients. However, in both groups, the outcome for those with an international prognostic index (IPI) score of 3-5, treated with the standard rituximab (R)-CHOP regimen, remains disappointing. Thus, in HIV-negative patients, studies are ongoing to determine whether chemotherapy intensification can improve prognosis. These more intensive chemotherapy regimens, such as CODOXM/IVAC, appear to be well tolerated in HIV-positive patients with Burkitt lymphoma, but their use in HIV-positive DLBCL has not been reported. **Aims.**To retrospectively review the response rate (RR), treatment toxicity and overall survival (OS) for HIV-positive patients with DLBCL, IPI score 3-5, and the role of treatment intensification. **Patients and Methods.** 50 patients from 5 UK centres treated with either R-CHOP (n=35) or CODOXM/IVAC+/-R (n=15) between 2004 and 2011 were included. The median age at diagnosis was 43 years (range 19-71; 38 male, 12 female).

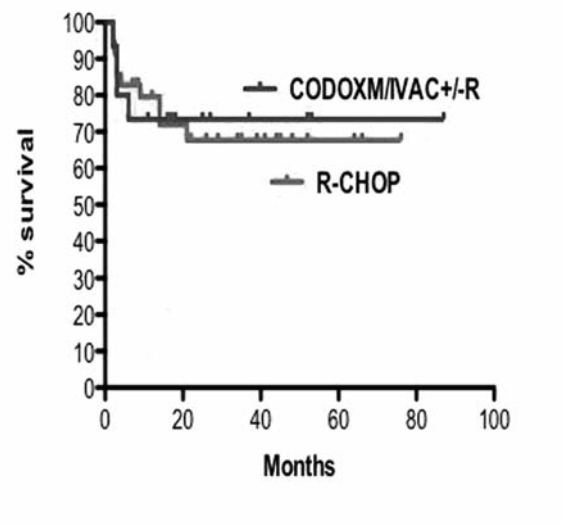

Figure 1. Kaplan Meier plot of survival for HIV-positive patients and high-risk DLBCL treated with R-CHOP vs CODOXM/IVAC+/-R.

Results. There were no significant differences in baseline characteristics of patients treated with the 2 different regimens. Nine patients were concomitantly diagnosed with HIV infection and DLBCL, and 21 (42%) were receiving cART at diagnosis. Seven patients (14%) had central nervous system (CNS) involvement. The median CD4 count and viral load at diagnosis was 91 cells/µl (range 0-740) and 40,633 copies/ml, respectively.Forty-seven patients (94%) received R with chemotherapy: median 6 doses (range 2-8). In those treated with R-CHOP and CODOXM/IVAC+/-R, the median number of cycles was 6 (range 2-8) and 4 (range 2-4), respectively. Additionally, 23 patients treated with R-CHOP received intrathecal methotrexate to prevent CNS recurrence. Seven patients with CNS disease received more intensive CNS-directed therapies. During treatment, 6 patients (17%) receiving R-CHOP and 7 (47%) receiving

CODOXM/IVAC+/-R required dose modifications. In conjunction with chemotherapy, 48 patients (96%) received cART. Additional, 48 (96%), 41 (82%) and 36 (72%) patients received prophylaxis against *pneumocystis jirovecii*, fungal infection and herpes viruses, respectively. Pre-emptive GCSF was administered to 100% of patients treated with CODOXM/IVAC+/-R and to 74% for R-CHOP.The RR and treatment-related mortality was not significantly different between the two groups. Overall, 34 patients (68%) achieved a complete remission, 4 (8%) a partial remission, 6 (12%) had no response and 6 (12%) died during treatment. There were more infections (p<0.0001) and grade 3/4 non-haematological (p=0.0004) toxicities in the CODOXM/IVAC+/-R treated group, and these patients required longer hospital admissions (p<0.0001).With a median follow-up of 28 months (range 7-87), the median remission duration and OS have not been reached, but these do not differ significantly between the 2 treatment groups (Figure 1). Two of 34 patients (6%) in whom CR was achieved have relapsed and 14 have died (8 lymphoma, 5 treatment-related infection).**Conclusions.** In our cohort, the outcome for HIV-positive patients with high-risk DLBCL is favourable (OS ~70% at 2 years). Treatment intensification with CODOXM/IVAC+/- R is feasible, but demonstrated no advantage over R-CHOP, instead resulting in increased morbidity and hospital stay.

0782

RITUXIMAB, BENDAMUSTINE AND LENALIDOMIDE IN PATIENTS WITH AGGRESSIVE B-CELL LYMPHOMA NOT ELIGIBLE FOR HIGH DOSE CHEMOTHERAPY OR ANTHRACYCLINE-BASED THERAPY -PHASE I RESULTS OF TRIAL SAKK 38/08

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Background. The majority of patients (pts) with aggressive B-cell lymphoma can be cured with R-CHOP-like standard first-line therapy. No standard therapy is established for pts who relapsed and are not eligible for intensive salvage regimens including high dose chemotherapy (HDT). In addition, frail pts are often not eligible for first-line anthracycline-based regimens. The combination of rituximab and bendamustine (RB) has demonstrated promising activity and tolerability anti-lymphoma treatment regimen. The immunomodulatory agent lenalidomide has also been shown to be active in the treatment of aggressive B-cell lymphoma. **Aims.** Based on the rationale of a hypothesised synergistic effect of lenalidomide in combination with the RB-regimen, this trial aims to develop a new effective and well tolerated regimen for pts with aggressive B-cell lymphoma not eligible for anthracycline-based chemotherapy or HDT. **Methods.** The phase I part was designed to identify the recommended dose (RD). The tentative RD was defined as one level below the dose level (DL) identifying ≥2/6 pts with a dose-limiting toxicity (DLT) during the first cycle. Lenalidomide was given at 10, 15 or 20 mg (dose level 1 to 3) on days 1-21 in combination with bendamustine 70 mg/m^2 days 1&2 and rituximab 375 mg/m^2 on day 1. Courses were repeated every 4 weeks. DLT was defined as one of the following events occurring within the first cycle: therapy related death, ≥ 6 missed doses of lenalidomide or delay of >2 weeks of cycle 2 due to trial drug-related adverse event (AE) toxicity, any grade 3 or 4 non-haematological AE related to trial treatment, neutrophils ≤0.5 x 10^9 G/L for ≥ 6 days, platelets ≤ 20 x 10^9 G/L or 21-50 x 10^9 G/L with major bleedings. Informed consent was obtained. **Results.** In the phase I part, 7 pts were enrolled between March 2010 and August 2011. Median age was 77 years (range 67- 79). WHO performance status 0 in 4 pts and 1 in 3 pts; disease stage I: 1 pt, stage II: 2 pts, stage III: 3 pts, stage IV: 1pt; B-symptoms: 2 pts; IPI 1 (2 pts); IPI 2 (4 pts); IPI 3 (1 pt). Two DLTs occurred at DL 2 within the first cycle: 1 pt had a delay of cycle 2 for > 2 weeks due to neutropenia grade 3, 1 pt experienced a grade 4 non-hematologic AE (myocardial infarction).Further non-dose-limiting grade 3/ 4 AE occurred during subsequent cycles: neutropenia grade 3 /4 (2 pts; DL 1), thrombocytopenia grade 3 (1pt; DL 2), febrile neutropenia grade 3 (1pt; DL 2), cardiac arrhythmia grade 3 (1pt; DL 2), gastrointestinal grade 4 (1pt; DL 2), neurologic-sensory grade 3 (1 pt; DL 1), dermatologic/skin grade 3 (1 pt; DL 2). **Conclusions.** The RD for the phase II part has been established at lenalidomide 10 mg/day for day 1-21 in combination with rituximab 375 mg/m^2 day 1 and bendamustine 70 mg/m^2 on day 1&2. Patient accrual is currently ongoing for the phase II part to evaluate the efficacy and safety of this regimen.

0783

FAVORABLE OUTCOME OF PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A SINGLE CENTER ANALYSIS

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Background. Among diffuse large B-cell lymphomas, primary mediastinal large B-cell lymphoma (PMBCL) represents a distinct entity to which a poor prognosis is frequently assigned. The optimal treatment is controversial, with an ongoing debate over the role of radiotherapy (RT) and of upfront autologous stem cell transplantation (ASCT). This retrospective study aimed at comparing the outcomes in PMBCL with CHOP +/- rituximab[R] + involved-field mediastinal RT in the pre- and post-rituximab era in a single center. **Methods.** Between October 1993 and April 2010, 60 consecutive patients (pts) with a diagnosis of PMBCL were registered. Median age was 28 years (range 19-70) and the majority of pts were female (65%) and had stage I/II (83%) bulky disease (88%); 42% had a high or intermediate-high International Prognostic Index. These characteristics did not differ significantly between the 44 pts who received first-line therapy with CHOP + RT (group A) and the 16 pts who received (since 2006) R-CHOP + RT (group B). **Results**. The complete remission (CR) rate with the first-line regimen was 70% (68% in group A *vs* 75% in group B); 7 further pts achieved CR with 2nd-line regimens, for a total CR rate of 82% (80 *vs* 87%, ns). Four relapses occurred (all in group A). Of 10 pts who received an ASCT for refractory or relapsed disease, only 4 achieved long-term disease-free survival (DFS). Late toxicity consisted of 2 cases of cardiomyopathy (1 death), 3 cases of pulmonary fibrosis and 1 thyroid carcinoma. In group A, with a median follow up of alive pts of 120 months, overall survival (OS) and DFS at 12 years were 58% and 70%, respectively. In group B (median follow up 52 months) OS and DFS were 87% and 100% at 6 years (ns for both between the 2 groups). **Conclusions**. In this single institution study, survival rates appear excellent since the addition of rituximab, with a trend to superior results in both DFS and OS. These encouraging results could be used to justify withdrawal of upfront RT, with the aim of reducing late side effects. In our view, given the relative inefficacy of ASCT salvage in PMBCL (in our series and others), consolidation RT is to be maintained until randomized studies prove otherwise.

0784

CLINICAL STUDY OF AUTOLOGOUS NATURAL KILLER CELLS COMBINED WITH RITUXIMAB FOR THE TREATMENT OF ELDERLY PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background. Antibody-dependent cellular cytotoxicity (ADCC) by natural killer (NK) cells is a major effector mechanism of the monoclonal anti-CD20 antibody rituximab in eliminating B-cell lymphomas. It is indicated that the combination of NK cells will enhance the efficiency of rituximab. And the combined regimen with low toxicity would be an option for the patients who cannot tolerate chemotherapy, especially for the elderly patients. **Aims.** In this study, the combination of NK cells with rituximab was applied to the elderly patients with diffuse large B-cell lymphoma (DLBCL) who cannot tolerate aggressive therapy with its associated toxicity, in order to evaluate the effectiveness and safety of the combined regimen in the elderly patients with DLBCL. **Methods.** 13 patients with DLBCL were enrolled, and the median age was 70 years old (range: 64-87). All of them have received 1 to 3 cycle chemotherapy of CHOP or RCHOP (rituximab on day 1 plus cyclophosphamide, doxorubicin, and vincristine on day 2, and prednisolone on day 2 through 6.). Seven patients were in partial remission (PR), four patients with stable disease (SD), and two patients with progression disease (PD) prior to study entry. The majority had an ECOG performance status at baseline of 1 or 2, and could not tolerate further aggressive chemotherapy. Written informed consent was obtained from each patient before therapy. Peripheral blood mononuclear cells (PBMC) were isolated by apheresis from the patients and used for expansion of NK cells in the medium containing IL-2, IL-12, IL-15 and monoclonal antibody against CD16 ex vivo in the GMP facility. Autologous NK cells (5.5-9.0 ×10^7/kg· d) were then infused back to individual patients on day 1 to 3 and rituximab 375 mg/m^2 on day 1 of each 21-day cycle for three to six cycles. Patients were assessed for the condition of remission, quality of life (QOL), and side effects every 3 months until progression. Clinical response and tolerability were examined according to international criteria. **Results.** The purity of NK cells (CD3-CD16+CD56+ cells) expanded ex vivo ranged from 78-97%. The lymphoma symptoms were reduced and QOL was improved in all patients. Overall response rate was 100% with 9 complete responders (69.2%), 4 partial responders (30.8%) after three to six cycles treatment. Seven patients had improved response from PR to CR and the two patients with PD after 1-2 cycles of RCHOP prior to study, got PR after three cycles of NK combined with Rituximab. Currently, one patient got CR, another stayed in PR after six cycles of treatment. Adverse events were

rare and mild. Two of the patients had fever. **Conclusions.** The regimen of autologous NK cell combined with rituximab is safe and efficacious for the treatment of elderly patients with DLBCL who cannot tolerate aggressive treatment. In our study, it is also implicated that NK cells potentially enhance the efficacy of rituximab against DLBCL and reverse the rituximab resistance. Thus this study encourages and warrants further evaluation of this immunotherapy for survival benefit.

0785

ALLOGENEIC STEM CELL TRANSPLANTATION AS CONSOLIDATION THERAPY AFTER CHOP-ALEMTUZUMAB CHEMOIMMUNOTHERAPY IN YOUNG UNTREATED PTCL PATIENTS

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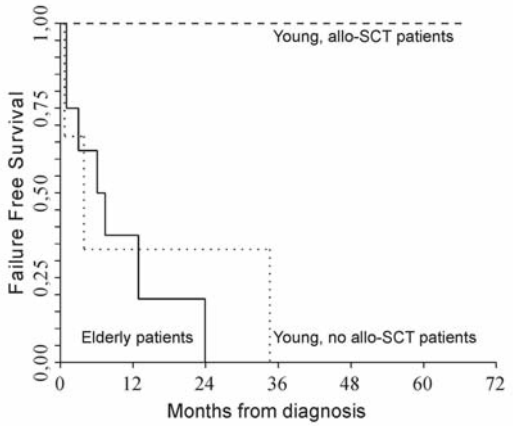
Background. Peripheral T-cell lymphoma (PTCL) is a group of disease characterized by widespread presentation, aggressive course, very poor response to chemotherapy and a 5-year OS ranging between 25% and 40%. Front-line autologous stem cell transplantation (ASCT) has been shown to produce only a modest increase in disease control with a 5-y OS not exceeding 50%. Allogeneic Stem cell transplantation (AlloSCT) proved to be useful mainly in relapsing or refractory PTCL lymphoma, and only anecdotic reports have addressed its role in first-line treatment. **Aims.** To explore the role of AlloSCT as consolidation therapy after induction chemoimmunotherapy with CHOP supplemented by Alemtuzumab (CHOP-C) in untreated PTCL. **Methods.** Sixteen PTCL patients (p) were consecutively admitted to our department from December 2005 to May 2011 with the following histology: 6 PTCL NOS, 3 Enteropathy associated T cell lymphoma (EATL), 5 Anaplastic Large-Cell lymphoma (ALCL) ALK-negative, 2 Angioimmunoblastic T cell lymphoma (AITL). P aged <60 were defined "young"and suitable for CHOP-C ± high-dose chemotherapy Cyclophosphamide, ARA-and Methotrexate (Hyper-C-HiDAM) and AlloSCT; p aged >60, defined "elderly"were treated with CHOP-C for 6 courses when fit for treatment, and with palliation if unfit.



Results Half of the p (8/16) were young (median age 46 range 23-60) with IPI 0 in 1p, 2 in 1p, 3 in 5p, 4 in 1p. After induction 5 (3 in CR 2 in PR) underwent AlloSCT from sibling (2) and unrelated (3) donor, after a thiotepa containing regimen. After a median follow-up of 23 months (11-67) all p are in continuous CR. Four patients had CMV reactivation, 1 had pneumonia from Enterococcus Faecium and Invasive Aspergillosis. Two EATL p. died +1, +5 months after diagnosis for progressing lymphoma. One p refused AlloSCT, treated after induction with ASCT relapsed +34 months after diagnosis. Half (8/16) classified as "elderly"(median age 72; range 66-83) had IPI 0 in 1p, 1 in 3p, 2 in 3p, 3 in 1p. Three completed 6 C-CHOP courses. One is in CR +9 month after diagnosis, 2 experienced early progression and died (+ 42 m + 19 m from diagnosis). Two progressed after the 2nd course and died (+2 m and +5m from d). One died for septic shock after the first C-CHOP; two aged 77 and 83 were treated with palliation and died (+4m and +9 after d). Two CMV reactivations and one sepsis were observed. After a median follow-up of 20 months (9-67), the median EFS and OS for the Allo-SCT p was not reached (EFS and OS 100%). For non-transplanted young p. and elderly p median EFS and OS were: 3.9, 4.6 and 6, 9 months, respectively (Median EFS shown figure 1). **Conclusions.** These data seem to support that although feasible, CHOP-C do not improve the disease outcome in elderly patients but is able to achieve response in a high percentage of young patients, bridging chemosensitive p straight to AlloSCT, with an higher disease control. These data, though very preliminary, need to be confirmed in a larger prospective cohort of patients.

0786

SAFETY AND ACTIVITY OF INTENSIVE SHORT-TERM CHEMOIMMUNOTHERAPY IN HIV-POSITIVE PATIENTS WITH BURKITT LYMPHOMA

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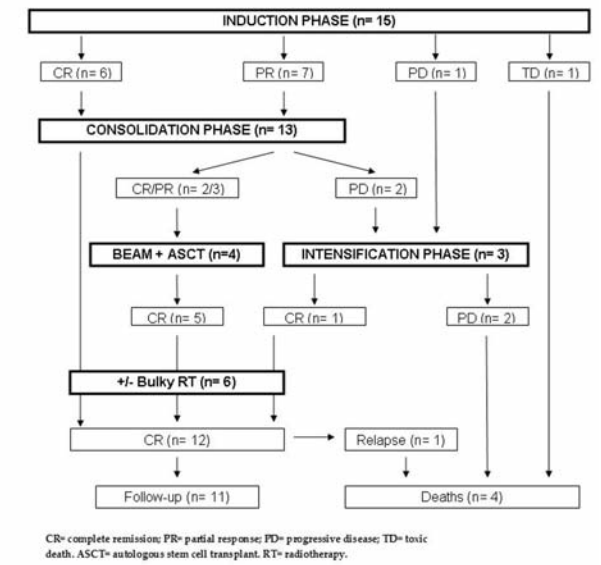
Background. Worldwide experience with intensive chemotherapy in HIV-positive patients with Burkitt lymphoma in post-HAART era is still limited, with complete remission (CR) rates of 70-80% and 2-year overall survival (OS) rates of 45-70%, but with a treatment-related mortality of 15-20%, mostly due to septic complications. Thus, we adapted an intensive, short-term chemoimmunotherapy program formerly used for HIV-negative patients to treat HIV-positive patients with Burkitt lymphoma with maintained efficacy and lower toxicity. **Aims.** To evaluate feasibility and activity of a new intensive, short-term chemoimmunotherapy program in HIV-positive patients with Burkitt lymphoma. **Methods.** Consecutive HIV-positive patients with Burkitt lymphoma, age ≤65 yrs and ECOG-PS ≤3 were treated with an intensified program at three Institutions. The program included a 40-day Induction Phase (*IP*) of sequential doses of methylprednisolone, cyclophosphamide, vincristine, rituximab, methotrexate, etoposide, and doxorubicin, with intrathecal prophylaxis/treatment. After *IP*, patients in CR received consolidation phase (*CP*; cytarabine + cisplatin or cytarabine + rituximab); patients in PR received *CP* followed by BEAM + ASCT; patients with SD/PD received intensification phase (R-ICEx2 => high-dose cyclophosphamide => high-dose cytarabine => BEAM + ASCT). Leukaphereses were performed after *CP*. Patients with residual or bulky disease at diagnosis were referred to involved-field radiotherapy. **Results.** Fifteen patients (median age 42 yrs, range 27-63; all males; ECOG-PS >1 in five cases) were considered. Most patients had advanced stage, increased LDH, B symptoms, bulky lesions, and extranodal disease (meningeal in two). Eight patients received HAART before lymphoma diagnosis; median CD4+ cells at lymphoma diagnosis was 248 (range 17-858).All patients but one completed *IP* (median duration 49 days; range 38-108), with >1 week delay in 7 patients. During *IP,* all patients experienced G3-G4 haematological toxicity, but it was manageable without drug dose reductions; only one patient showed G4 non-haematological toxicity (transient diarrhea). Response after *IP* was CR in six patients and PR in seven (ORR= 87%; 95%CI: 70-100%); one patient experienced meningeal PD and one died of septic complications (Figure).



CR= complete remission; PR= partial response; PD= progressive disease; TD= toxic death. ASCT= autologous stem cell transplant. RT= radiotherapy.

Thirteen patients were referred to *CP*; the first four patients received cytarabine+cisplatin combination and experienced prolonged G4 neutropenia, bacterial infections and viral reactivations (CMV); the following 9 patients were

treated with cytarabine+rituximab combination; none of them experienced infectious events. Leukaphereses were successful in 9 of the 11 referred patients. Four patients required BEAM treatment and six were referred to radiation therapy.Response after the whole treatment was CR in 12 patients (CRR= 80%; CI: 60-100%). At a median follow-up of 22 months (range 6-30), 11 responsive patients remain disease-free, whereas the remaining responder experienced relapse at 8 months, with a 2-year PFS of 71%. Eleven patients are alive, three patients died of lymphoma and one of sepsis, with a 2-year OS of 70%. **Conclusions.** This intensive, short-term chemoimmunotherapy is feasible in HIV-positive patients with Burkitt lymphoma and shows a better tolerability profile and a similar activity if compared to other more demanding and resource-consuming regimens. The addition of rituximab seems to not increase the risk of septic complications. A multicenter prospective phase II trial is ongoing.

0787

A RUXOLITINIB INDIVIDUAL SUPPLY PROGRAM FOR PATIENTS WITH PRIMARY MYELOFIBROSIS (PMF), POST-POLYCYTHEMIA VERA MYELOFIBROSIS (PPV-MF), OR POST-ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS (PET-MF)

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Background. Ruxolitinib is a potent and selective JAK1/2 inhibitor approved in the United States based on results of the phase 3 COMFORT trials. Ruxolitinib demonstrated rapid and durable reductions in splenomegaly and improved MF-related symptoms and quality of life for patients with MF. Because of the unmet medical need, ruxolitinib has been made available through an individual patient supply program (IPSP) outside the United States.

Table. Demographics and Patient Characteristics by Request Status[a]

Characteristic	Approved Patients (n=746)
Sex, n (%)	
Male	433 (58)
Female	310 (42)
Median age, y	67.0
Risk factor, n (%)	
High risk	276 (37)
Intermediate 2 risk	260 (35)
Intermediate 1 risk	142 (19)
Low risk	27 (4)
JAK mutation, n (%)	
+	486 (74)
–	143 (22)
ECOG PS, n (%)	
0/1	137 (18)/352 (47)
2/3	137 (18)/17 (2)
Splenic irradiation, n (%)	
Yes	65 (9)
No	576 (77)
Transfusion	
Yes	309 (41)
No	339 (45)
Transplant candidate	
Yes	25 (3)
No	710 (95)
Spleen size (cm)	
n	706
Median (range)	17.0 (0-40.0)
Splenectomy	
Yes	11 (2)
No	276 (37)

[a] Numbers may not add up to 100% because of missing data

Methods. Patients with PMF, PPV-MF, or PET-MF, who are in need of treatment as determined by their physicians, are evaluated for eligibility, irrespective of JAK2 mutation status. The starting dose of ruxolitinib is determined on the basis of baseline platelet count (15 or 20mg twice daily for patients with platelet counts of 100-200x10^9/L and >200x10^9/L, respectively) and can be adjusted for efficacy and safety. Dose changes during treatment are registered, and significant AEs and serious AEs (SAEs) are monitored.To date, 811 requests have been received from >530 physicians in 38 countries, including Canada, Australia, and locations in Europe, Latin America, the Middle East, and Asia. Baseline characteristics for patients whose requests for access were approved/enrolled (n=746) are shown in Table. Drug resupply requests are generally received every 3 months; follow-up data are available for 213 (54.6%) of the 390 patients with sufficient time on the program. Of these, 164 (77%) remain on ruxolitinib therapy, 19 (9%) have discontinued, 6 (3%) died, and 24 (11%) did not initiate therapy. Spleen response (change from enrollment to resupply) was available for 95 patients in whom spleen length increased, decreased, or was unchanged in 3, 80, and 12 patients, respectively. Changes in constitutional symptoms were available for 60 patients. Symptoms were reported to decrease in 43 patients and increase or remain unchanged for 1 and 16 patients, respectively. Safety information was available for 108 patients, of whom 34 were noted as having a significant AE or SAE by the investigators at the time of resupply.To date, the proportion of patients with the *JAK2*V617F mutation enrolled in this IPSP (74%) is higher than that for the general MF population (50%-60%) and may reflect the preference of physicians to treat more *JAK2*V617F-positive patients with ruxolitinib, even though ruxolitinib has demonstrated efficacy in both patient types (Verstovsek S, et al. *N Engl J Med*. 2010;363(12):1117-1127). **Conclusions**. Ruxolitinib, the only drug to have completed phase 3 studies, has garnered substantial requests for access through the IPSP underscoring the need for an effective medical treatment in patients over a range of IPSS risk-assessment scores. With the exception of a higher number of *JAK2*V617F mutation-positive patients, characteristics of the IPSP patients are generally similar to those expected in the overall MF patient population. Response and safety patterns seen in the IPSP appear to be similar to those from the COMFORT trials.

0788

LYPOSOMAL CYTARABINE IN THE TREATMENT AND PROPHYLAXIS OF CNS LYMPHOMA - LONG TERM RESULTS OF RESULTS OF 120 PATIENTS TREATED IN PLRG CENTERS

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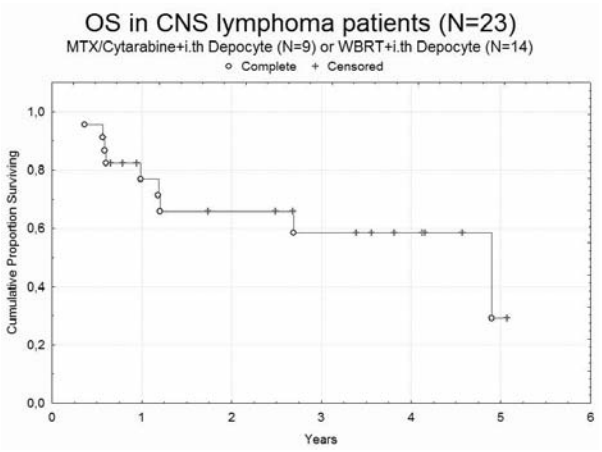
Background. Treatment and prophylaxis of Central Nervous System (CNS) lymphoma remains a therapeutic challenge. Liposomal Cytarabine (DepoCyte) is a sustained release formulation of Ara-C for intrathecal administration that maintains cytotoxic concentrations in the CSF for more than 14 days.This is a retrospective analysis of 120 patients treated according to the PLRG protocols including DepoCyte. **Methods.** The efficacy and safety of Liposomal Cytarabine (DepoCyte) was assessed in three cohorts of patients: one in prophylaxis (N=88) and two in treatment (N=32). In our series prophylaxis was regarded necessary in lymphoblastic or Burkitt lymphoma (N=13) and high risk cases of other aggressive lymphomas (DLBCL, PMBCL, PTCL) defined as involvement of specific sites (infiltration of vertebral column, orbits, sinuses or testis; N=17) or at least 2 of recognized risk factors (IPI 3-5, elevated LDH, 2 or more extranodal sites; N=75). None of those patients had neurological symptoms at diagnosis, and the average pleocytosis was 2,4 (range 0-10). The patients received 3, 1 (range 1-6) DepoCyte injections every 2-4 weeks at the time of systemic chemotherapy or after its completion. In the treatment cohort we identified 9 patients with an increased pleocytosis (39, range 15-174) due to lymphoma in cytological analysis without neurological symptoms or typical radiologic findings. Those patients received systemic chemotherapy in doses not penetrating to CNS and the 3, 9 (range 2-7) DepoCyte injections with curative attempt. The third cohort consisted of 23 patients with a measurable CNS disease in imaging studies and/or important neurological signs (14 cases of PCNS lymphoma and 9 cases systemic lymphoma with CNS infiltrations. Those patients were treated with standard chemotherapy (whole brain radiotherapy -14 cases or high doses of cytarabine/methothrexate-9 cases) and additional Depocyte injection (4,3 doses, range 2-8). Both adverse reaction and survival data (PFS, CNS PFS, OS) were monitored during the average observation time of 3 years. **Results.** Liposomal Cytarabine was well tolerated (56% patients had no side effects, 37% experienced transient grade 1-2 adverse events: headache, nausea and vomiting, dizziness or fever, 7% developed grade 3 headaches or fever). At the average observation time of 3 years, none of 88 high risk lym-

phoma patients relapsed in CNS after Lyposomal Cytarabine prophylaxis. In patients with cerebrospinal fluid infiltration without neurological symptoms or radiologic findings Depocyte as the only CNS directed treatment was efficient in preventing relapse in 8/9 cases. The addition of Liposomal Cytarabine to standard radiotherapy or high dose cytarabine/metothrexate based chemotherapy in 23 lymphoma cases with neurological symptoms and infiltrates visualized in imaging studies possibly increased OS (median OS - 4,8 years, Figure 1). **Conclusions.** Liposomal Cytarabine administered nntrathecaly has good toxicity profile. Its pharmacokinetics allows using it at the time of routine chemotherapy without the necessity of additional hospital admissions.1) It is effective in CNS prophylaxis of high risk aggressive lymphoma patients,2) It may be considered adequate as the only CNS treatment in asymptomatic cases with increased pleocytosis,3) It possibly increases the efficacy of radiotherapy/systemic chemotherapy in cases with brain infiltrations.



Indolent lymphomas

0789

BRAFV600E NEGATIVE HAIRY CELL LEUKEMIA IS RARE AND NOT ASSOCIATED WITH SPECIFIC IGHV REARRANGEMENTS OR A SPECIFIC IGHV MUTATION STATUS

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Background. The BRAFV600E mutation has initially been discovered in all cases of hairy cell leukemia (HCL), but not in cases of variant HCL (HCLv). Recently, this perfect correlation has been challenged by studies reporting HCL cases without BRAFV600E. Interestingly, the immunoglobulin heavy chain variable region gene IGHV4-34, which has been associated with poor prognosis in HCL, appeared exclusively and to a high percentage in these BRAFV600E-negative cases of classic HCL and also in HCLv (Xi et al., Blood, 2011). **Aims.** We wanted to characterize our cohorts of HCL and HCLv for the presence of BRAFV600E and correlate the results with IGHV gene usage. **Methods.** We analyzed the bone marrow or peripheral blood of 52 cases with HCL and 12 cases with HCLv at diagnosis as confirmed by multiparameter flow cytometry (MFC). The BRAFV600E mutation was detected by an mRNA-based reverse transcription allele-specific real-time quantification (RQ-PCR) assay. Results are given as %BRAFV600E/BRAFwt. IGHV genes and mutation status were analyzed by the use of Biomed-2 primers. An identity of >= 98% of the analyzed IGHV sequence compared to published germline sequences was considered an unmutated IGHV status. **Results.** In our cohort the median percent leukemic cells was 16% (range 0.2 - 74%) for HCL and 33% (range 5 - 59%) for HCLv as determined by MFC. The BRAFV600E mutation was detected in 51/55 (92%) of HCL cases, whereas 0/12 of HCLv were positive. The median BRAFV600E expression ratio of positive cases was 32.9 (range 3.9 - 280.3) and that of all negative cases was 0.005 (range 0.000 - 0.009). In detail, the percent leukemic cells in the four HCL BRAFV600E negative cases were 0.2%, 8%, 28% and 66% by MFC and within the clone size that can be detected with the BRAFV600E-specific RQ-PCR. Next, the IGHV genes and the IGHV mutation status of all samples were analyzed. We detected an unmutated IGHV status in 10/55 (18%) of HCL, which was less frequent compared to 5/12 (42%) of HCLv ($p = 0.087$). We could not find the usage of the IGHV4-34 gene, which has been associated with poor prognosis and BRAFV600E negativity in HCL, in any of our classic HCL cases, but in 5/12 (42%) of the HCLv cases. The IGHV mutation status was unmutated in 4/5 (80%) IGHV4-34 cases (100% identity to germline each). The four cases of classic HCL, which lacked BRAFV600E expression, expressed the IGHV genes IGHV1-3*01 (96.5% identity), IGHV1-69*02 (94.0% identity), IGHV3-9*01 (96.9% identity) and IGHV6-1*01 (99.0% identity), which were also expressed by BRAFV600E positive HCL cases in our cohort. **Conclusions.** We confirm the use of BRAFV600E as a marker for HCL, which helps to delineate classic HCL from the variant, prognostically poor form. We do, however, find rare HCL cases that lack the BRAF mutation, a finding that has been only appreciated by few of the recent studies. In our cohort, the BRAFV600E negative cases were not associated with a specific IGHV gene or IGHV mutation status and IGHV4-34 was only detected in HCLv but not in classic HCL.

0790

RANDOMIZED PHASE II TRIAL COMPARING OBINUTUZUMAB (GA101) WITH RITUXIMAB IN PATIENTS WITH RELAPSED CD20+ INDOLENT B-CELL NON-HODGKIN LYMPHOMA: PRELIMINARY ANALYSIS OF THE GAUSS STUDY

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Background. GA101 (obinutuzumab) is a unique glycoengineered type II anti-

CD20 monoclonal antibody in phase II/III clinical trials for chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma (NHL). In preclinical models, GA101 enhanced direct cell death and increased antibody-dependent cell-mediated cytotoxicity compared with other anti-CD20 antibodies. GA101 clinical studies have demonstrated responses in patients with relapsed/refractory NHL and CLL however, this is the first head-to-head study to directly compare clinical responses of GA101 with rituximab. **Aims.** This randomized Phase II trial compares the safety and efficacy of monotherapy with GA101 vs. rituximab in patients with relapsed indolent NHL. **Methods.** Patients with relapsed CD20+ indolent NHL requiring therapy who had demonstrated a prior response to a rituximab-containing regimen lasting ≥6 months were eligible. A total of 175 patients (149 follicular lymphoma [FL] and 26 non-follicular indolent NHL) stratified by histology and country were randomized 1:1 to receive 4 weekly infusions of either GA101 (1000 mg, n=88) or rituximab (375 mg/m^2, n=87). End-of-treatment response was assessed 28-42 days after the last induction dose. Patients without evidence of progression following induction therapy received maintenance GA101 or rituximab q2m for up to 2 years at the same dose. The primary endpoint was the overall response rate (ORR) at the end of induction in the FL population as assessed by investigators and was designed to detect a 15% difference between GA101 and rituximab with ~80% power for a 1-sided chi-square test at a significance level of 0.2.

Table 1. ORR at end of induction and best ORR by investigator and IRF assessment in FL patients.

	Investigator-assessed		IRF-assessed	
Response, n (%)	rituximab (n=75)	obinutuzumab (n=74)	rituximab (n=75)	obinutuzumab (n=74)
Overall response rate (ORR)	25 (33.3)	33 (44.6)	20 (26.7)	33 (44.6)
CR/CRu	4 (5.3)	9 (12.2)	3 (4.0)	4 (5.4)
PR	21 (28.0)	24 (32.4)	17 (22.7)	29 (39.2)
Difference in ORR, % [95% CI]	11.3 [–5.1, 27.6]		17.9 [2.0, 33.8]	
p-value*	0.08		0.01	
Best ORR (BORR)	48 (64.0)	49 (66.2)	35 (46.7)	45 (60.8)
CR/CRu	14 (18.7)	26 (35.1)	15 (20.0)	20 (27.0)
PR	34 (45.3)	23 (31.1)	20 (26.7)	25 (33.8)
Difference in BORR, % [95% CI]	2.2 [–13.9, 18.3]		14.1 [–2.5, 30.8]	
p-value*	0.39		0.04	

* One-sided, chi-squared test with a significance level=0.2
CR = complete response, CRu = unconfirmed complete response, PR = partial response

Results. The clinical cutoff date was September 1, 2011, with a median observation time of 15 months for both arms. Overall, patients in the two treatment groups were balanced for baseline characteristics. Investigator-assessed ORR for patients in the GA101 arm was higher (44.6% [33/74]) than for the rituximab arm (33.3% [25/75]) in the FL population (difference 11.3% 95% CI: -5.1-27.6; *p*=0.08) (Table 1). A blinded independent review facility (IRF) also assessed responses (Table 1): ORR for GA101 was higher (44.6% [33/74]) compared with rituximab (26.7% [20/75]) constituting a difference of 17.9% (95% CI: 2.0-33.8; *p* = 0.01). The median PFS is not different between the treatment arms at this point in time.No new safety signals were observed in either arm. The most common adverse events (occurring in ≥5 patients) were infusion-related reactions (IRRs) (GA101 vs. rituximab: any grade, 74% vs. 51%), fatigue (25% vs. 20%), cough (21% vs. 6%) and upper respiratory tract infection (8% vs.10%). The majority of grade 3/4 adverse events reported over the entire treatment period occurred as a result of IRRs (GA101 vs. rituximab: 11% vs. 6%). **Conclusions.** This is the first head-to-head trial of GA101 against rituximab and has demonstrated higher responses rates without appreciable differences in safety. Although a higher rate of IRRs and cough was noted with GA101, the majority were grade 1/2 in severity and did not result in significant differences in treatment discontinuation. GA101 is under study in Phase III trials in combination with chemotherapy.

0791

FIRST-LINE ANTHRACYCLIN-CONTAINING THERAPY FOLLOWED BY AUTOLOGOUS HSCT AT RELAPSE IS THE BEST SEQUENCE OF TREATMENTS IN FOLLICULAR LYMPHOMA. A MULTICENTER STUDY BY THE FONDAZIONE ITALIANA LINFOMI

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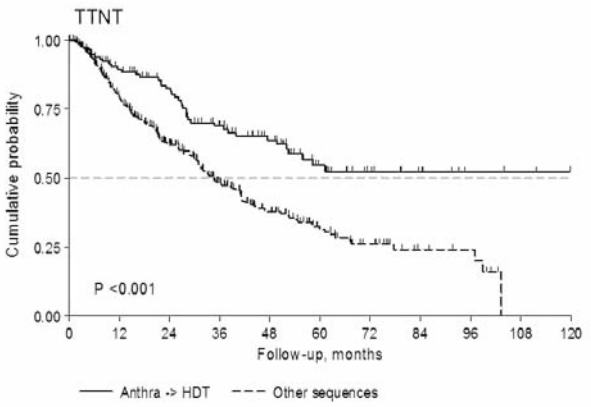
Background. The disease course of follicular lymphoma has been usually characterized by frequent remissions and subsequent relapses, with progressively shorter treatment-free intervals. During the course of the disease, patients often receive multiple lines of active treatments ranging from single-agent chemotherapy (CHT) or monoclonal antibodies, to high-dose therapy with stem cell transplantation (HSCT). In relapsed patients the efficacy of subsequent treatments may be affected by the type and the intensity of the previous treatments. However it is currently not known whether a definite sequence of active treatments exists which optimizes the outcome of patients and randomized trials can hardly be designed to answer this question. **Aims**. The REFOLL study is an observational, multicenter, retrospective study launched by the Fondazione Italiana Linfomi (FIL) to analyze the impact of different combinations of first- and second-line treatments on patient's outcome. **Methods.** Of 582 patients with follicular lymphoma at first relapse between 2000 and 2008 registered from 25 Institutions, 546 were analyzable. They had received either alkylating- (AA) (22%), or anthracyclin- (AC)(61%) or nucleoside analogues-based (NA)(17%) therapy as first-line treatment, with rituximab (R) in 52%. AA patients were older (P<.001) but had less stage III-IV (P=.011), FLIPI high-risk disease (P=.02), and had received maintenance R more often (P<.001) compared to other groups. Second-line treatment options included the same first-line options w/wo R (AA: 20%; AC 18%; NA: 14%), autologous HSCT (29%), or monoclonal antibody only (19%). The primary endpoint was time to next treatment (TTNT) after first relapse.



Results. The median follow-up from first relapse was 40 months (range 1-125). TTNT ranged between 1 and 120 months (median 23 months). AC+/-R as first-line treatment was associated with a better TTNT (HR: 0.71, P=0.007) than

AA+/-R and NAI+/-R. Among second-line treatments, auto-HSCT performed better when compared to any other second-line treatment with or without R (HR ranging from 1.79 to 2.40). Interestingly, using multiple Cox regression, the TTNT after auto-HSCT was significantly better in patients receiving AC as first line treatment (HR ranging from 1.88 to 3.3 compared to any other sequence with or without R) (Figure 1). Other factors independently influencing TTNT after HSCT were R maintenance (HR: 0.66), duration of first remission > 12 months (HR: 0.8), and stage III-IV at diagnosis (HR: 1.89). Overall survival from diagnosis was 89% at 5-yr (CI95% 85-91%) and the sequences CHT->HSCT and CHT->CHT with R showed a better outcome than CHT ->CHT without R (HR:0.55, P=0.015). Adjusted by age and stage at diagnosis the superiority becomes marginal (HR 0.61, P=0.047). **Conclusions.** Auto-HSCT at relapse obtained the best TTNT compared to standard CHT or chemoimmunotherapy. The results of auto-HSCT were optimized by the use of anthracyclin-containing CHT as first line treatment rather than of other types of CHT whereas the use of rituximab in first-line did not impact on the efficacy of auto-HSCT. This study supports the concept that sequences of similarly active treatments do not necessarily obtain similar results, which is important in the management of indolent diseases like follicular lymphoma and requires further, possibly prospective, studies.

0792

LONG-TERM FOLLOW-UP ANALYSIS OF A SERIES OF 97 PATIENTS WITH SPLENIC MARGINAL ZONE LYMPHOMA TREATED WITH SPLENECTOMY AS A FIRST LINE TREATMENT

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Background. Splenic marginal zone lymphoma (SMZL) is an indolent B-cell lymphoma involving the spleen.SMZL spreads to the bone marrow and the blood where circulating villous lymphocytes are usually found. When required, several therapeutic options may be proposed as first line without consensus, except for HCV-positive patients. **Aims.** To perform a large retrospective analysis of 97 patients treated by splenectomy as a first line treatment. **Methods.** We collected patients with SMZL treated in two centers from June 1997 to February 2011. All of them were HCV negative. At diagnosis, median age was 63 years (range, 29-87). Sex ratio M/F was 0.78. Most of the patients (77%) had a good performance status. Involvement of bone marrow and blood was found in 88% and in 73% of the patients, respectively. High LDH level was seen in 46% of the patients. High aaIPI (> 2) was found in 48% (n=27) patients. The Intergruppo Italiano Linfomi (IIL) score was 0 for 30% (9), 1 for 40% (12), and 2 for 30% (n=9) of the patients. Anemia less than 12 and thrombocytopenia less than 100 occurred in 33% (n=32) and in 12% (n=12) of the patients, respectively. A monoclonal component was found for 33 patients. The most common associated immunological event was an autoimmune haemolytic anemia and / or a positive coombs' test. **Results.** Median follow-up after splenectomy was 6.3 years. Complications after splenectomy occurred in 5% of the patients, and included 2 splenic vein thrombosis, 1 pancreatitis, 1 nosocomial pneumopathy, 1 post-operative ARDS. Adjuvant therapy (clb, fludarabine, CHOP, R-CHOP, or rituximab alone).was proposed to 20% (20) of the patients.One year after splenectomy, only 4% of the patients had anemia (range 8.9 to 11.7 g/dL). None of the patients had thrombocytopenia. The 5- and 10-year overall survival (OS) rates were of 84% and of 67%, respectively. The respective 5- and 10- year progression free survival (PFS) rates were of 62% and 47%. Half of the dead patients (11%, n=11) died because of a non illness-related event (pulmonary infections, stroke, myocardial infarction, other non-related malignancies, accident). Histological transformation to high grade lymphoma occurred in 11% (11) of the patients.Significant prognostic factors for OS were age at diagnosis more than 60 years (p= .0382) and a histological transformation after splenectomy (p= .0328). Significant prognostic factors for PFS were an initial bone marrow involvement (p= .0436) and a histological transformation (p= .0001). None of the prognostic scores, neither aaIPI nor IIL score, discriminated significantly the outcome of the patients. **Conclusions.** Splenectomy proposed as first line therapy in patients with SMZL is an appropriate and safe treatment. It allowed to normalize cytopenias during the first year and is associated with a good PFS.

0793

INOTUZUMAB OZOGAMICIN IN PATIENTS WITH INDOLENT B-CELL NON-HODGKIN LYMPHOMA REFRACTORY TO RITUXIMAB AND CHEMOTHERAPY OR RADIOIMMUNOTHERAPY

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Background. Inotuzumab ozogamicin (INO) is a humanized anti-CD22 antibody conjugated with calicheamicin, a potent cytotoxic antitumor antibiotic. **Aims.** This phase 2 study evaluated the safety and efficacy of INO in indolent B-cell non-Hodgkin lymphoma (B-NHL) refractory to rituximab and chemotherapy, or radioimmunotherapy. **Methods.** Patients with follicular (FL), marginal zone (MZL), or small lymphocytic lymphoma (SLL) received INO 1.8 mg/m^2 every 28 days for 4 to 8 cycles, with dose and/or frequency adjusted based on toxicities. Patients must have ≥2 prior systemic therapies and no response/progression within 6 months of completion of rituximab-containing therapy or within 12 months of radioimmunotherapy. **Results.** Eighty-one patients have been enrolled and dosed with INO: 72 with FL, 4 with MZL, and 5 with SLL. Median age was 63 years (range, 29-84 years). Patients were heavily pretreated: median of 3 prior anticancer regimens (range, 1-14; 35% had ≥4), 5/81 [6%] patients had prior autologous stem cell transplant, and 29/79 [37%] were refractory to their most recent prior chemotherapy. Based on FLIPI scores, 24%, 28%, and 49% of FL patients were low, intermediate, and high risk, respectively. Median follow-up was 10.1 months. Patients received a median of 3 INO doses (range, 1-8 doses), with dose adjustments due to adverse events (AEs) allowed: 17% had reductions, and 15% had delays. The most common grade 3/4 AEs were thrombocytopenia (57%) and neutropenia (32%). Among 39 patients with grade 3/4 thrombocytopenia and complete laboratory data, mean time to grade 1/0 recovery was 35.9 days (range, 3.0-176.0 days). The most common non-hematologic grade 3/4 AEs were nausea (5%), elevated gamma-glutamyltransferase (5%; 2/5 resolved), elevated aspartate aminotransferase (4%; all resolved), and pneumonia (4%). In total, 41% patients discontinued treatment due to AEs, including 28% due to persistent thrombocytopenia that failed to recover to grade 1/0 within the 28-day dose delay allowed per protocol. Among 75 evaluable patients, the objective response rate (ORR) was 61% (FL, 67%) and complete response rate was 28% (FL, 32%); median duration of objective response was 24.8 months. Based on FLIPI scores, ORRs in FL patients were 75% in low-risk patients, 58% in intermediate-risk patients, and 56% in high-risk FL patients. ORR was 19% (5% complete response) for patients with bulky disease (≥7.5 cm) and 58% (35% complete response) for patients refractory to their most recent chemotherapy. Median progression-free survival (PFS) was 12.0 months (FL, 26.9 months; MZL, 8.8 months; SLL, 2.4 months). Among FL patients, median PFS has not been reached in low-risk patients, with a 1-year PFS rate of 76%; median PFS for intermediate- and high-risk patients were 11.1 and 12.6 months, respectively. Median overall survival has not been reached for all patients or FL patients; 1-year overall survival rate in FL patients was 84%. Median overall survival for MZL and SLL patients was 11.3 and 9.9 months, respectively. **Conclusions.** In patients with indolent B-NHL refractory to rituximab plus chemotherapy or radioimmunotherapy, INO demonstrated notable clinical activity with durable responses, especially in FL patients. Hematologic, gastrointestinal, and hepatic AEs were the most common toxicities.

0794

FINAL RESULTS OF STAGE 1 OF A PHASE IB STUDY TO INVESTIGATE THE PHARMACOKINETICS (PK), SAFETY, AND TOLERABILITY OF SUBCUTANEOUS RITUXIMAB IN FOLLICULAR LYMPHOMA (FL) AS PART OF MAINTENANCE TREATMENT

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Background. Rituximab is currently administered by intravenous (IV) infusion. Subcutaneous (SC) administration could significantly simplify treatment and reduce administration time. To determine a rituximab SC dose that yields a non-inferior serum trough concentration (C_{trough}) compared with rituximab IV (375 mg/m^2), a two-stage, adaptive Phase Ib study was conducted. In Stage 1, FL patients with a response to R-based induction therapy and at least one IV rituximab dose in maintenance were randomized to receive a single dose of rituximab at 375 mg/m^2 IV, 375 mg/m^2 SC, 625 mg/m^2 SC, or 800 mg/m^2 SC. Patients then continued with IV treatment (375 mg/m^2) on a 2-monthly (q2m) or 3-monthly (q3m) schedule until completion of 2 years of maintenance. However, once a final SC dose had been elucidated, patients randomized to an SC cohort who had completed 1 year of maintenance therapy were given the option to receive remaining maintenance doses with rituximab SC. In Stage 1 of this dose-finding study, IV and SC PK data were integrated into a PK model, and model-based simulations were then used to predict serum C_{trough} and AUC for various rituximab SC fixed doses (1100-1400 mg). **Aims.** To describe the model-based simulations used to determine a dose of rituximab SC that yields a non-inferior serum C_{trough} to that of the standard dose of rituximab IV given q2m or q3m. To provide an update on previously reported (ASH 2010) data on safety and tolerability of rituximab SC in the maintenance setting for FL.

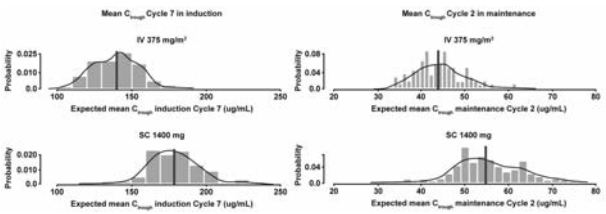

Figure 1. Distribution of simulated mean Ctrough levels comparing IV (375 mg/m^2) and SC (1400 mg) at Cycle 7 of induction and Cycle 2 of maintenance.

Methods. Population PK analysis was conducted using non-linear mixed-effect modeling with NONMEM VI. The first-order conditional estimation method with interaction was used. The structural model was a two-compartment model comprising a time-varying clearance component. SC injection was characterized by the SC absolute bioavailability and the rate of absorption from the SC compartment. After model qualification, simulations were used to compare SC C_{trough} and AUC values with those of IV for various rituximab SC doses during the maintenance and induction period. **Results.** Model-based simulations were performed for a virtual population of at least 50 patients in each arm for both maintenance and induction periods and were repeated 100 times. The outcome suggested that a dose of 1400 mg rituximab SC would potentially maintain patients at a level of efficacious concentrations independent of the regimen.Figure 1 illustrates the distribution of predicted mean C_{trough} at Cycle 7 in induction and Cycle 2 in maintenance, comparing the IV dose of 375 mg/m^2 with the fixed SC dose of 1400 mg. At the selected dose of 1400 mg, mean C_{trough} values are predicted to be non-inferior to those achieved with the IV dose. Similar simulations were performed for the 3-monthly maintenance regimen as well as for AUC_{tau} in both induction and maintenance. **Conclusions.** A fixed dose of 1400 mg has been selected for formal C_{trough} non-inferiority testing in Stage 2 of the trial. Treatment with SC doses of rituximab was well tolerated, with an AE profile comparable to that of rituximab IV. These data support further testing of rituximab SC.

0795

CLINICOPATHOLOGICAL FEATURES OF EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF THE MUCOSA ASSOCIATED LYMHOID TISSUE (MALT LYMPHOMA)

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Background. Extranodal marginal zone B-cell lymphoma of the mucosa associated lymphoid tissue (MALT lymphoma) is among the more common types of lymphoma and is thought to follow a clinically indolent course. Recently, it has been suggested that the rate of dissemination might be substantial, and that systemic therapies are beneficial also in patients with localized disease. **Aims.** To retrospectively assess clinicopathological features and outcome of different types of therapy along with defining potential prognostic parameters in all patients with MALT lymphoma staged and treated at a single institution (University referral center). **Methods.** Between 1999 - 2011, a total of 245 patients with MALT lymphoma were diagnosed and treated at our institution, who constitute the collective analysed. Interestingly, only 35% of patients had gastric lymphoma, while 65% had extragastric lymphomas. All patients had undergone uniform staging, and the following parameters had been assessed: age, gender, localisation and stage, infection with Helicobacter pylori (HP), Hepatitis A, B and C, presence of an autoimmune disease, plasmacytic differentiation, monoclonal gammopathy, factors included in the IPI including LDH, beta-2-microglobulin, MALT-lymphoma specific translocations including t(11;18)(q21;q21), type of treatment, response to therapy, relapse/progression, time to relapse/progression and survival. **Results.** After an interim analysis of 180 patients (76 male/104 female), there was no difference in terms of survival between gastric versus extragastric MALT lymphomas, while a lower IPI-score (0,1 versus higher) was significantly associated with better survival (p= 0.014). after a median follow-up of 52 months (IQR 20 - 93), 16% have died. Curiously, low IPI-scores had a significantly shorter time to progression (141 vs 155 months, p=0.017) and a significantly higher rate of relapse as compared to higher IPI. Patients with gastric MALT lymphoma had similar rates of disseminated disease as extragastric MALT lymphomas (p=0.289, but stage was neither associated with survival nor relapse or time to progression. In total, 38% had only antibiotic therapy, 35% chemo/immunotherapy, 12% radiation and 9% surgery as initial treatment, while 5% had only wait and see after initial diagnosis. There was no influence of extragastric disease versus gastric lymphoma, LDH, B2MCG, plasmacytic differentiation and monoclonal gammopathy on relapse/progression and time to relapse. In addition, an underlying autoimmune disease (seen in 33% of patients) did not influence clinical outcome. Patients with gastric lymphoma, however, had a significantly higher likelihood to require further therapy than patients with extragastric lymphoma, most probably reflecting a selection bias of HP-refractory patients in our referral center. Patients undergoing systemic therapy (immuno/chemotherapy) had a signifcantly longer time to progression/relapse than patients after radiotherapy (117 vs 71 months, p = 0.006), further underscoring the potential of systemic therapy even in localised stages. However, there was no difference in survival according to initial therapy, suggesting a high rate of successful salvage in relapsing patients. **Conclusions.** MALT lymphoma has an excellent prognosis irrespective of initial therapy, localisation and stage, suggesting that patients should be initially managed with non-toxic therapies. However, early application of systemic therapies appears to be associated with a longer time to relapse also in localized disease.

0796

ADDITION OF RITUXIMAB TO INVOLVED-FIELD RADIOTHERAPY PROLONGS PROGRESSION FREE SURVIVAL IN STAGE I-II FOLLICULAR LYMPHOMAS: A MULTICENTRIC, RETROSPECTIVE SURVEY

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Background. Radiotherapy (RT) is considered the standard treatment for stage I-II, non bulky, Follicular Lymphoma (FL). Despite initial therapy, most patients relapse especially outside the radiation field. To reduce the risk of disease recurrence, many authors have suggested to extend the irradiation field or to combine systemic chemotherapy to RT. However, both these approaches are associated with increased risk of early and late toxicity. Thus, the use of involved-field (IF) RT remain widely employed in stage I-II FL. The anti-CD20 monoclonal antibody rituximab (RIT) is extensively used in FL, with proven efficacy and excellent tolerability, either alone or in combination with chemotherapy. It should be effectively combined with RT, as well. **Aims.** To evaluate retrospectively the safety and efficacy of rituximab, administered in combination with RT as initial treatment for localized FL. **Methods.** From July 1999 to June 2011, 43 consecutive patients were treated for grade I-II FL, with stage I-II non-bulky at five Italian Hematologic centers. The treatment included 4 weekly infusions of rituximab at a dose of 375 mg/m^2, followed by IF-RT. Main patient characteristics were: median age = 50 years (range: 34-82); M/F ratio=26/17; FLIPI 0/1 = 35/8; Bulky = 0; stage I / II = 37/6. The median dose of RT was 30.6 Gray (Gy) (range 20-40). Bone marrow PCR to detect molecular disease was performed in 32 patients: 10 (31%) were PCR positive.

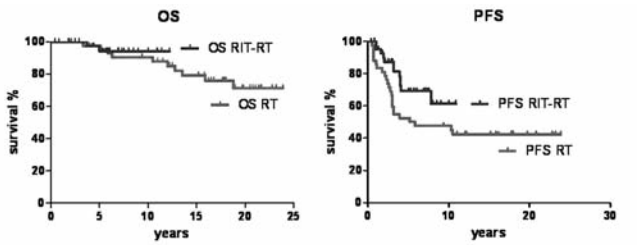

Figure 1. Overall survival (OS) and progression free survival (PFS) of patients treated with Rituximab and involved-field radiotherapy (RIT-RT) and patients receiving only radiotherapy (RT). OS p=NS; PFS p=0.032.

Results. the treatment was generally well tolerated, the addition of rituximab did not result in significant side effects except for some mild allergic reactions at the first infusion. All patients responded to therapy, with 42 (98%) complete remissions (CR) and 1 (2%) partial remission (PR). At a median follow up of 6.4 years (range: 0.4 to 12.2), the PFS was 69%, and overall survival (OS) 95%. As of December 2011, 41 of 43 patients (95%) were alive and 30 (70%) are in continuous CR; there were two deaths at 3 and 4 years for lung cancer in two smoker patients; 12 patients (30%) recurred (6 were PCR+ at diagnosis, 4 PCR- and 2 had no probe). These results were compared with a matched historical group of 46 patients with similar clinical characteristics and treated with IF-RT alone. The median PFS of RT alone vs RIT-RT was 5.2 years versus not yet reached. As shown in Figure 1, the 5-years PFS of RT alone vs RIT-RT was 52% and 69% respectively(p=0.032); the 5-years OS was similar 92% and 94%. **Conclusions**. Rituximab followed by RT-IF is a well tolerated treatment for patients with stage I-II FL; this approach offers prolonged PFS, higher than that observed in subjects treated with RT alone. The long-term results with RIT-RT are similar to those reported with the combination of RT to chemotherapy, but with significantly reduced side effects. The results suggest the usefulness of randomized phase III study designed to compare prospectively RIT-RT vs. RT alone in limited-stage FL.

0797

A PHASE II STUDY OF LENALIDOMIDE IN PATIENTS WITH EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF THE MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT-LYMPHOMA)

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Background. MALT lymphoma ranks among the more common lymphoma entities, and shares certain features with multiple myeloma. In view of this and the activity of lenalidomide in various B-cell lymphomas we have initiated a phase II study of lenalidomide in patients with MALT-lymphoma. **Aims.** To evaluate the efficacy of lenalidomide in patients with MALT lymphoma. **Patients and Methods.** Patients with histologically verified advanced MALT lymphoma (in case of gastric MALT lymphoma with demonstrated refractoriness to HP-eradication, i.e. at least a 12 months follow up after successful HP-eradication) were included in the study. Treatment consisted of lenalidomide 25 mg p.o. days 1 - 21, with a 7 day-break after each cycle. Restaging was scheduled after 3 and 6 courses, respectively. Response was assessed by radiological criteria or in case of gastric MALT by applying the GELA criteria for histological response.The study had been approved by the Ethical Board of the Medical University of Vienna and before opening the trial, it had been registered at www.clinicaltrial.gov. Informed consent was obtained of all patients. **Results** A total of 18 patients were included in the trial (10 female/8 male); 5 had gastric and 13 had extragastric MALT lymphoma (7 orbital, 3 pulmonary, 1 parotid, 1 intestinal and 1 subcutaneous); 4 patients had an underlying autoimmune disease (2 had Sjögren's disease, 2 a chronic autoimmune thyroiditis). In eleven of these patients lenalidomide was applied as the first line therapy.Sixteen patients were evaluable, showing an overall response rate of 62% (10/16; 6 complete and 4 partial remissions). Four patients had stable disease, while two progressed, including one case of transformation to diffuse large B-cell lymphoma. Side effects were manageable and included neutropenia (WHO grade III in 3 patients) as the leading hematotoxicity and pruritus/exanthema of the skin as the leading non-hematologic side effect (8 patients, 7 grade I/II). One patient each had to be hospitalized for pneumonia and pruritus/exanthema, respectively, but both recovered after consecutive dose reduction of lenalidomide to 15 mg. After a median follow-up of 18 months, one patient has died from lymphoma, while the remaining are alive and relapse-free. **Conclusions.** These data suggest activity of lenalidomide monotherapy in MALT lymphoma with an overall response rate of 62% and manageable toxicity in this study.

0798

IN VIVO PURGING FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA: UPDATE OF A PROSPECTIVE PHASE II STUDY

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Background. High dose chemotherapy and autologous stem cell transplantation (ASCT) has been shown effective in the long-term control of relapsed/refractory follicular lymphoma. It has also been reported that the achievement of bcl-2 negative status is associated with a lower risk of recurrence. **Aims.** To evaluate the clinical outcome of patients with relapsed or refractory FL treated with immunochemotherapy, *in vivo* purging and high-dose therapy with ASCT. **Methods.** We updated a prospective multicenter phase 2 trial (*Arcaini et al. Annals of Oncology, 2008*) and herein we report data on 124 relapsed/refractory FL pts treated from 1999 to 2010 with a program of anthracycline-based debulking chemotherapy , immunochemotherapy (2-4 R-COP),

in vivo purging and SC mobilization with HD-AraC and rituximab followed by ASCT conditioned with BEAM. In selected pts 2 rituximab doses were administered on days +14 and +21 post infusion. Response was assessed according to the International Working Group Response criteria (Cheson et al. JCO 1999). **Results.** Median age was 52 yrs; grade 3 FL histology was detected in 15% of pts. Median number of previous chemotherapy lines was 1 (range 1-4). Prior radiotherapy was administered in 23 pts. Ann Arbor stage was III-IV in 77% and BM was involved in 49% pts. FLIPI was low in 48%, intermediate in 32%, high in 20%; FLIPI2 was low in 10%, intermediate in 70% and high in 20%. PB bcl-2 was positive in 32/67 pts and BM bcl-2 was positive in 51/106. Debulking chemotherapy allowed to obtain CR in 11% and PR in 79%, and molecular remission in 54% (28/52) of informative patients for bcl-2. After R-COP, CR was obtained in 58% and PR in 35%. 3 pts did not proceed to mobilization for progression. Before mobilization, 40 out of 45 informative pts for bcl-2 in BM (89%) became negative and 29/33 (88%) in PB. Two pts were poor mobilizers and 1 did not proceed for infection; 118 pts successfully mobilized (median number of CD34+ cells collected 14.6 x 10^6/Kg) and 117 proceeded to ASCT (1 was diagnosed with lung cancer before ASCT). All harvests in molecularly informative pts were bcl-2 negative. The median number of infused CD34+ was 7.86 x 10^6/Kg and the median take was on day +12 from BEAM. TRM was 0%. For the whole series the 5-yr PFS is 54% (median 7.2 yrs) and the 5-yr OS is 85% (median not reached) (Figure 1). After a median follow-up of 5.2 yrs (range 0.5-12.6 yrs), 81 pts are still in CR. Absence of the bcl-2 rearrangement in PB and BM after ASCT is associated with persistent clinical remission. In 3 pts an histologic shift was documented after ASCT and 4 pts developed a myelodisplastic syndrome; 16 pts died (3 in CR). **Conclusions**: these updated data confirm that prolonged PFS is achievable in relapsed/refractory pts with *in vivo* purging and ASCT. Persistent molecular response is associated with a better outcome.

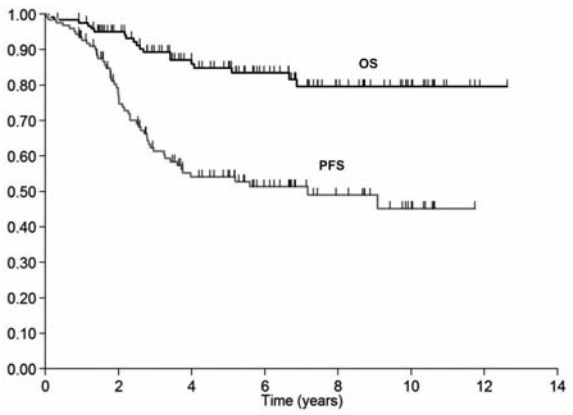

Figure 1. OS and PFS of entire series.

0799

INOTUZUMAB OZOGAMICIN PLUS RITUXIMAB VERSUS DEFINED INVESTIGATOR'S CHOICE IN CD20+/CD22+ FOLLICULAR NON-HODGKIN LYMPHOMA

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Background. CD22 is expressed on the majority of B-cell non-Hodgkin lymphomas (NHL), including follicular NHL (FL). Inotuzumab ozogamicin (INO) is an anti-CD22 antibody conjugated to calicheamicin, a potent antitumor cytotoxic antibiotic. Rituximab (R), approved for FL treatment, can enhance the activity of chemotherapy. **Aims.** The primary objective was to evaluate progression-free survival (PFS) of R-INO versus a defined investigator's choice therapy. The study was terminated early due to poor recruitment, enrolling only 29 patients: 15, Arm 1 (all treated); 14, Arm 2 (13 treated). **Methods.** This open-label, phase 3 study was designed to enroll 978 patients with CD20+/CD22+ FL treated with 1-2 prior R-containing regimens. Patients were randomized 1:1"'Arm 1: R 375 mg/m^2 (Day 1) plus INO 1.8 mg/m^2 (Day 2), every 28 days; Arm 2: investigator's choice of R-CVP (R 375 mg/m^2, cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2 [Day 1], prednisone 40 mg/m^2 [Days 1-5]) or R-FND (R 375 mg/m^2 [Day 1], mitoxantrone 10 mg/m^2 [Day 2], fludarabine 25 mg/m^2 [Days 2-4], dexamethasone 20 mg/day [Days 1-5]). Patients were stratified by number of prior regimens, investigator's choice of therapy, and geographic region. Patients received up to 8 cycles.**Results.** For enrolled Arm 1 and Arm 2 patients, respectively: median ages, 66 years and 61.5 years; 60% and 43% male; 67% and 79% Caucasian. Median treatment durations were 143 days (range, 30-228 days) and 152 days (range, 111-203 days). Seven patients in each arm completed the treatment phase.Although a small sample size, the hazard of disease progression or death in Arm 1 appeared less than Arm 2 (hazard ratio=0.19; 95% CI, 0.04-1.02; *P*=0.036; Cox proportional hazard model, controlling for stratification factors). Arm 1 had higher PFS rates (12-month, 86% vs 55%; 24-month, 74% vs 27%); median PFS was not estimable in Arm 1 and 16.4 months in Arm 2. Objective response rate was 93% in Arm 1 versus 64% in Arm 2. The 24-month overall survival rates in Arms 1 and 2 were 87% versus 67%, respectively. Two (13%) patients in Arm 1 and 4 (29%) in Arm 2 died. Fewer patients in Arm 1 versus Arm 2 experienced grade 3/4 treatment-related adverse events (AEs) of neutropenia (20% vs 77%), leukopenia (7% vs 39%), febrile neutropenia (0% vs 23%), pneumonia (0% vs 8%), and anemia (0% vs 8%); other grade 3/4 AEs (³10% of patients) were thrombocytopenia (40% vs 23%) and lymphopenia (13% vs 8%). Treatment-related serious AEs were reported for 4 patients in Arm 1 (2 with hepatobiliary disorders) and 6 patients in Arm 2 (5 with hematologic disorders, 3 with pneumonia). Five patients in Arm 1 (none in Arm 2) discontinued treatment due to an AE (neutropenia, increased GGT, hyperbilirubinemia, pyrexia, venoocclusive disease). **Conclusions.** In this terminated study, R-INO showed longer PFS and a higher response rate than investigator's choice (R-CVP/R-FND) in these FL patients. Each arm had different, but acceptable, safety profiles. These interesting results need to be confirmed in a larger trial. Further clinical trials of R-INO for NHL treatment are ongoing.

0800

EFFECTIVENESS OF FIRST-LINE CHEMOIMMUNOTHERAPY REGIMENS FOR PATIENTS DIAGNOSED WITH FOLLICULAR LYMPHOMA (FL) IN THE US: DATA FROM THE NATIONAL LYMPHOCARE STUDY (NLCS)

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Background. Our initial publication of the NLCS identified that rituximab (R) with chemotherapy constitutes more than half (51.9%) of strategies used in newly diagnosed FL patients (pts) in theUS. Given the lack of data comparing the effectiveness of front-line R-chemotherapy regimens in clinical practice, we examined outcomes for pts with stage III/IV FL receiving R with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), R with cyclophosphamide, vincristine, and prednisone (R-CVP), or R with a fludarabine-based regimen (R-Flu), as first-line therapy **Aims.** Assess the progression-free survival (PFS), time to next treatment (TTNT), and overall survival (OS). Determine the effect of each treatment regimen on these outcomes stratified by FL grade and FLIPI risk.

Table 1. Two-year Kaplan-Meier estimates, PFS, TTNT and OS for stage III/IV patients and selected subgroups.

Two-Year Survival Probability									
	2-Year PFS Rate			2-Year TTNT Rate			2-Year OS Rate		
	R-CHOP	R-CVP	R-Flu	R-CHOP	R-CVP	R-Flu	R-CHOP	R-CVP	R-Flu
Overall	0.77	0.71	0.76	0.77	0.72	0.78	0.94	0.88	0.91
All Stage III/IV Patients, HR (95% CI)									
	R-Flu vs R-CVP			R-CHOP vs R-CVP					
	PFS	TTNT	OS	PFS	TTNT	OS			
Overall[1]	**0.61 (0.38-0.98)**	**0.42 (0.25-0.70)**	0.72 (0.35-1.47)	0.82 (0.60-1.13)	0.90 (0.64-1.26)	0.64 (0.39-1.04)			
Stage III/IV Patient Subgroups, HR (95% CI)[2]									
	PFS	TTNT	OS	PFS	TTNT	OS			
FLIPI high risk[3]	**0.54 (0.31-0.95)**	**0.37 (0.18-0.77)**	0.52 (0.26-1.06)	**0.66 (0.45-0.96)**	0.86 (0.57-1.32)	**0.38 (0.23-0.63)**			
Grade 1-2[4]	**0.55 (0.32-0.94)**	**0.36 (0.20-0.63)**	0.60 (0.25-1.43)	0.94 (0.65-1.36)	0.99 (0.68-1.44)	0.75 (0.42-1.33)			

[1]Adjusted for FLIPI components, histology, use of R-maintenance, practice setting, bone marrow involvement, sex, and geographic region.
[2]G3 patient subgroup was not evaluated due to small sample size.
[3]Adjusted for use of R-maintenance, practice setting, bone marrow involvement, sex, and geographic region.
[4]Adjusted for FLIPI components, use of R-maintenance, practice setting, bone marrow involvement, sex, and geographic region.

Methods. The NLCS is a prospective, multicenter, observational study collecting data on previously untreated pts with FL diagnosed from 2004 to 2007 at 265 sites in theUS. Initial management decisions were made by the treating physician. Two-year Kaplan-Meier estimation was used to evaluate PFS, TTNT, and OS. To compare the impact of treatment regimen on PFS, TTNT, and OS, Cox proportional hazards models were used controlling for FLIPI components, community or academic practice setting, R-maintenance or observation following treatment, and additional factors that were significantly correlated with induction regimen (bone marrow involvement, sex, and geographic region). **Results.** Of 2727 evaluable pts, 901 had stage III/IV FL and went on to receive the aforementioned regimens of interest (575 grade 1-2 [G1-2] FL; 206 grade 3 [G3] FL, 120 mixed or unknown grade). 51% of G1-2 pts received R-CHOP (n=296), 32% received R-CVP (n=186), and 16% received R-Flu (n=93). Among G1-2 pts, 241 had high-risk FLIPI scores (49% R-CHOP, 39% R-CVP, and 12% R-Flu). In comparison with G1-2 pts, more G3 pts received R-CHOP (82%, n=169), with R-CVP and R-Flu treatments given to 13% (n=27) and 5% (n=10) pts, respectively. Among G3 pts, 91 had high-risk FLIPI scores (79% R-CHOP, 17% R-CVP, 3% R-Flu).With a median follow-up of 62 months, median PFS, TTNT, and OS were not reached in most cases. Results of the Kaplan-Meier estimation and adjusted Cox proportional hazards models for PFS, TTNT, and OS are presented in the table. Two-year response rates for PFS, TTNT and OS were comparable across regimens. PFS and TTNT increased in pts treated with R-Flu vs. R-CVP overall (HR=0.61 and HR=0.42, respectively), and in G1-2 pts and pts with high-risk FLIPI. The benefit of R-Flu could not be evaluated in G3 pts due to small sample size. Although patients receiving R-CHOP tended to have slightly better outcomes than those receiving R-CVP, the only significant difference was seen in the high-risk FLIPI group (HR=0.66 and HR=0.38 for PFS and OS respectively). **Conclusions.** First-line R-chemotherapy regimens in clinical practice in the US provide robust PFS, TTNT, and OS. R-Flu is associated with significantly longer PFS and TTNT overall and for patients in selected sub-groups when compared with R-CVP. For high-risk FLIPI patients, R-CHOP was associated with improved PFS and OS compared with R-CVP.

0801

IRON CONTRIBUTES TO BORTEZOMIB SENSITIVITY IN MULTIPLE MYELOMA CELLS

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Background. Multiple Myeloma (MM) is a malignant still incurable plasma cell disorder. Pharmacological treatment with the proteasome inhibitor bortezomib has improved patient outcome. However, bortezomib-resistance remains a major clinical problem. In the attempt to improve bortezomib efficacy, we explored whether cellular iron status may contribute to resistance. Proteasome plays an important role in iron homeostasis that might be altered by proteasome inhibition inducing iron excess. Excess iron is potentially toxic through generation of reactive oxygen species (ROS) that may cause cell death. To avoid ROS generation, cellular iron homeostasis is tightly regulated by the coordinated expression of iron import (Transferrin Receptor 1, TfR1), export (ferroportin) and storage (ferritins) proteins. **Aims.** To identify novel approaches to sensitize bortezomib-resistant cells. **Methods.** We analyzed iron and oxidative status together with cell viability in 7 MM cell lines (MMc) and in plasma cells from 4 patients. Cells were treated with increasing bortezomib concentrations with or without iron supplementation. Manipulation of ferritin levels was obtained by two approaches: by drug- (deferoxamine, DFO) induced iron reduction and by silencing with shRNA technology. **Results.** MMc showed a range of responsiveness to bortezomib. By plotting sensitivity versus iron parameters and ROS scavenger proteins we found that bortezomib resistance directly correlates only with ferritin levels and thus with iron storage capacity. In MMc with low ferritin, bortezomib enhanced ROS generation and iron supplementation increased bortezomib sensitivity by increasing ROS levels. In contrast, iron toxicity was less evident in bortezomib-resistant cells with high ferritin levels. In the absence of bortezomib, all MMc showed the expected coordinated regulation of iron proteins in response to iron. They were able to reduce iron import protein TfR1 while increasing ferritin levels and iron storage capacity after iron supplementation. Bortezomib deregulated the coordinated expression of iron proteins. In particular, it caused a two-fold increase of TfR1 through protein stabilization and inhibited iron storage by blocking translation of new ferritin molecules, without affecting iron export. In this way bortezomib increased iron toxicity. We concluded that basal ferritin levels mediate the sensitivity to iron toxicity in bortezomib-treated cells.To further prove this conclusion we manipulated ferritin levels in resistant cells. DFO treatment strongly abolished ferritin expression, while silencing by shRNA reduced ferritin of about 75%. In both cases the reduction of basal ferritin levels before bortezomib treatment sensitized resistant MMc.On patient derived CD138+ cells, we confirmed that iron supplementation sensitizes cells to bortezomib, that bortezomib prevents ferritin increase and that DFO pre-treatment overcomes bortezomib resistance. **Conclusions.** Bortezomib increases iron toxicity by deregulating the coordinated expression of iron proteins and by increasing cell iron entry in the presence of defective iron storage. Thus iron supplementation enhances bortezomib sensitivity in both MMc and patient primary cells. Ferritin reduction is a mechanism to increase iron toxicity overcoming bortezomib resistance worth to be explored *in vivo*.

0802

ANTIVIRAL THERAPY(AT) FOR THE PATIENTS WITH INDOLENT B-CELL NON-HODGKIN LYMPHOMAS (IBCNHL) ASSOCIATED WITH CHRONIC HEPATITIS C. HEMATOLOGICAL RESPONSE DETERMINING BY IMMUNOGISTOCHEMISTRY ANALYSIS

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In a number of researches it is shown that the virus of a hepatitis C is one of etiopathogenic factors of NHL. Last time a number of lymphoproliferative diseases has been also related to the virus associated with tumors. According the data of WHO classification in 2008 HCV infection takes part in etiopathogenesis of several indolent lymphoms and those tumors may be virus associated.

Hematological response after antiviral therapy (AT) is the strongest argument in favourof an oncogenic role of hepatitis C virus (HCV) in non-Hodgkin's B-cell lymphoma (NHL)Method: In Cancer Research Center named by N.N Blokhina in the period 2000-2011 we observed 556 patients with NHL, who along with diagnosed of tumor were also analysed for chronic viral hepatitis C infection. In our research we analysed hematological and virological response of 87 pts with IBCNHL who had markers of HCL infection. Patients with IBCNHL were distributed in 2 groups: 1 group included 54 pts whose tumoral cells was positive of immunochemistry by proteins of hepatitis C (IGH+), and 2 group -33pts with negative immunochemistry tumoral cells (IGH-). Of those 87 patients in 24 patients the disease was diagnosed in I-II stage, in 63 - в III-IV stage Age of the patients varied from 27y.o. to 70 y.o. 65 patients had ALT and AST to be over normal. Mediana was 2,5 normal. Level of increasing of ALT achieved 10 normal. In IGH+ group level of HCV RNA was from $3x10^3$ to $7x10^7$ c/ml, in IGH- group level RNA was from negative to $5x10^5$ c/ml. All pts were characterized by an indolent course of disease not needing immediate conventional anti-lymphoma therapy. 74 pts received interferon (IFN) every days 3 m.U with ribavirin and 13 received only INF Results. 7 pts discontinued AT for toxicity, all pts IGH- group No pts interrupted AT for IBCNHL progression. Pts treated with AT in IGH+ group achieved a CR 37(69%) and 13 (24%) a PR while 4 had stable disease. Median F-UP of 3.3 yrs. Pts treated with AT in Igh- group achieved only PR in 13 (39%) a while 20 had stable disease. Virological response (VR) was achieved in 68 pts (78%). Considering the IGH+ group treated with AT , hematologic response (CR + PR) was highly significantly associated to the achievement of a VR (p <0.001)Conclusions: These data in a large series of pts confirm high rates of lymphoma regression in group of pts with indolent NHL IGH+ with AT. For this pts antiviral therapy may be defining as a first line therapy Lower response rate in second group suggested as a first line therapy anti-lymphoma treatment.

0803

PHARMACOECONOMIC EVALUATION OF RITUXIMAB AS MAINTENANCE TREATMENT FOR FOLLICULAR LYMPHOMA: RESULTS OF A REAL WORLD POPULATION BASED STUDY

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Background. Follicular lymphoma (FL) is the largest subtype of indolent non-Hodgkin lymphoma. FL is incurable and median overall survival ranges from six to ten years. The EORTC 20981 trial revealed longer progression-free survival (median 3.7 versus 1.3 years) after treatment with rituximab maintenance compared to observation. While randomised controlled trials (RCTs) are the golden standard for establishing effectiveness, the generalizability in daily clinical practice is limited. Therefore, health policy makers and clinicians increasingly require information on outcomes in unselected patient populations which are not treated under the idealised circumstances created in RCTs. **Aims.** Using real world data, we evaluate cost-effectiveness of rituximab maintenance compared to observation in FL patients responding to second line chemotherapy. **Methods.** A population based registry was created including patients with non-Hodgkin lymphoma diagnosed in the Netherlands since 2004. We selected patients with follicular lymphoma who responded to second line chemotherapy. Data was collected from medical patient records on patient characteristics, resource utilisation, treatment information (e.g. regimens, dosages and number of cycles) and treatment outcomes (e.g. response and survival). A cost-effectiveness model was applied to calculate cost per life year gained (LYG) and quality adjusted life year (QALY). **Results.** Our database contained 1457 patients with non-Hodgkin lymphoma including 545 patients diagnosed with FL. The observation group consisted of 46 patients responding to second line chemotherapy. The maintenance group included 33 patients receiving rituximab maintenance after second induction treatment. Median age was 60 [Range 34-82] and 62 years [Range 30-92] in the observation and maintenance group and not significantly different. Differences between the two groups were found for response to prior treatment (p=0.032) and prior treatment with rituximab in the second line (p=0.004). Besides, non-significant differences for B-symptoms and distribution of the FLIPI score were found. Compared to the EORTC 20981 trial, patients in the registry were older (p=0.0006) and although substantial variation across patients was observed, dosage, number of cycles and duration of treatment were comparable to the trial. We investigated overall survival (OS) and this was significantly better in the maintenance group (p = 0.021) compared to the observation group, although median OS was not reached. Incremental cost per LYG and QALY were low with € 5,156 per QALY and € 5,246 per LYG. Extensive sensitivity analyses did not change the results. While the results in our study are based on real world data and will be more generalizable to daily clinical practice, both OS and costs should be interpreted with caution since observed dissimilarities between the observation and maintenance groups suggested that the groups represent different types of patients. **Conclusions**. FL patients in daily practice are not identical to the patients in the EORTC 20981 trial emphasising the importance of studying real world data. However, analysing non-randomised patient groups is challenging because of the differences between the observation and maintenance group and therefore results should be interpreted carefully. However, we do find that in this real world setting, cost-effectiveness ratios for maintenance treatment with rituximab are low compared to other haematological treatments.

0804

PRE-EMPTIVE RITUXIMAB-BASED TREATMENT OF MOLECULAR RELAPSES IN FOLLICULAR AND MANTLE CELL LYMPHOMA

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Background. Achievement of maximal cytoreduction is a major goal of anti-lymphoma therapy: several studies indicate that follicular (FL) and mantle-cell lymphoma (MCL) patients entering molecular remission (MR) detected by PCR-based minimal residual disease (MRD) methods benefit of superior outcome independently from age and treatment received [Ladetto, Blood 2008; Pott, Blood 2010]. Conversely, the occurrence of molecular relapse (M-rel) often anticipates subsequent clinical relapse. Andersen et al. [JClinOncol2009] reported successful MR reinduction following rituximab administration in MCL patients, but no similar experience has ever been reported in FL. **Aims.** We here collected retrospective cases receiving at our Institutions pre-emptive treatment for M-rel with rituximab (pRTX) to evaluate feasibility and efficacy of this approach in FL and MCL. **Methods.** Between 2000-2008, FL and MCL patients in complete clinical response experiencing M-rel underwent pRTX. MRD was assessed on bone marrow using Bcl-2/IGH (FL) and Bcl-1/IGH translocation or IGH rearrangement (MCL), as described [Ladetto,BiolBloodMarrowTransplant2006;Ladetto,Blood2008]. According to the Nordic group approach, treatment decision was based on nested-PCR results; real-time quantitative (RQ)-PCR analysis was performed in all cases with available DNA leftovers. M-rel was defined as recurrence after MR of PCR-positivity on two consecutive samples (confirmed by direct sequencing), in absence of clinical relapse; molecular persistence (M-per) as persistent PCR-positivity after chemoimmunotherapy; MR reinduction as the occurrence of two consecutive PCR-negative samples within one year after pRTX; pRTX consisted of four weekly rituximab infusions: a double course was given within six months to patients still PCR-positive after the first course. Additional pRTX courses were offered, at the discretion of the treating physician, to patients experiencing subsequent M-rels. **Results.** Eighteen patients received at least one pRTX course (nine FL, nine MCL; median age 52 years; range 32-68; 16 males). These patients were previously treated with rituximab-supplemented autologous transplantation (13 patients) or R-CHOP and experienced either M-per (six patients) or M-rel (twelve patients, median time from treatment 40 months, range 10-103). Overall, 24 pRTX procedures (14 in MCL and ten in FL patients) were performed (17 single, seven double), with no grade 3-4 toxicities recorded; eighteen for first M-rel or M-per and six for second or third M-rel. In 18 cases MR reinduction was successful while in six failed (four failures in MCL, two in FL; five as first pRTX, one as second pRTX). None of the 14 patients reentering MR relapsed within two years from pRTX while two relapses were observed within six months among the six pRTX failures. Four second M-rels (occurring in FL patients) did not receive pRTX retreatment and relapsed within one year. RQ-PCR was performed on 80% of samples, basically confirming nested-PCR results; the majority of M-rels showed low MRD levels (median: 3x10^-4, range: 10^-6 to 8x10^-2). In this small series we failed to find associations between tumor burden at M-rel and pRTX efficacy. **Conclusions.** pRTX was safe and effectively reinduced MR in 75% of procedures in both FL and MCL, including cases of repeated delivery. Prospective MRD-tailored trials are needed to verify the clinical benefit of this approach, particularly in the context of maintenance regimens.

0805

LONG TERM RESULTS OF THE LNH- PRO TRIAL FOR PATIENTS WITH INDOLENT NON HODGKIN´S LYMPHOMA TREATED WITH CVP PLUS INTERFERON ALFA 2B (IFN)

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Background. Indolent non-Hodgkin B-cell lymphomas are clonal mature B-cell proliferations for which a curative treatment has not been defined to date. Nevertheless, survival has significantly improved since the incorporation of agents with immunomodulatory activity, such as recombinant alpha2 interferon (IFNα2b) first and anti CD-20 antibody (rituximab) in the last decade. Currently, there is not a consensus about the best chemotherapy to be associated with and treatment strategies under study use progression free survival as a surrogate marker for survival, but updated long term results are frequently lacking. Since 1990 our group introduced, for naïve low-grade NHL, the use of IFN in the induction treatment in association with CVP, using the Bagley regimen which includes 2 gr/m^2 of Cyclophosphamide per cycle. Herein, we report the long term results of our trial. **Aims.** . To evaluate the long term outcome of 170 patients treated with CVP plus IFN, 71 patients included in the LNH- PRO multicenter trial(Arranz et al, JCO 1998) and 99 patients in the subsequent trial that evaluated the role of maintenance IFN (Cannata el al, ASH 2004). **Methods.** From 1990 to 2000, 170 patients received CVP plus IFN. Doses were Cyclofosfamide 2g/m^2 po, Prednisone 500mg/m^2 po, Vincristine 1.4mg/m^2 (max 2mg) and IFN 3 MU/m^2 sc, 3 times a week for 12 weeks. Patients received the number of cycles necessary to achieve maximum response. Radiotherapy to bulky areas was admitted. Efficacy analysis has been performed on an intention to treat basis and data were updated in March 2011.

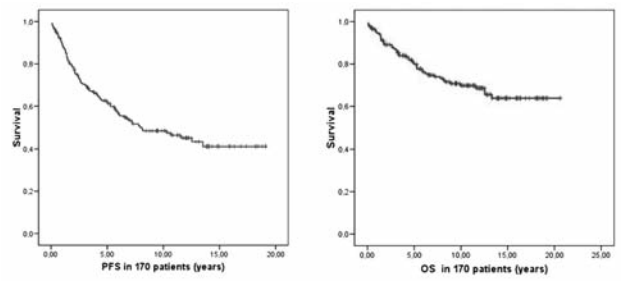

Figure 1. PFS and OS in the series.

Results. Included entities were grade 1-2 FL 65%, SLL 21% and MZL 13%. Patient's characteristics were median age 56 years-old (22-78 years-old), stage III-IV 80%, bone marrow involvement 58%, and 31% of high-risk FLIPI among FL patients. Median number of cycles administered was 6 (range 1-15). Overall response rate was 87%, with 68% achieving CR (116 patients). After a median follow-up of 10 years for surviving patients (range 0.3-19.6 years), median PFS for the whole series was 7.9 years (95% CI: 3.7-12.09 years, Fig 1) and 10 years for FL patients (95% CI: 4.5-15.9 years). DFS is 54 % (± 0.5% SD). Actuarial OS at 20 years is 64 % (± 5% SD, Figure 2). A plateau in PFS, DFS and OS curves is observed after 13 years.Fifty one patients (30%) have died due to disease progression (31 patients), secondary neoplasms (7 patients), cardiopathy (4 patients) and unknown causes (9 patients). Secondary neoplasms have been diagnosed in 24 patients (13%). All were solid tumours, except for 1 myelodisplastic syndrome. **Conclusions.** CVP chemotherapy plus 12 weeks IFN is a very effective therapy that has changed the expected natural history of our indolent NHL. Median PFS of nearly 8 years and OS of 64 % at 20 years should be considered for future strategies in the treatment of indolent NHL. In order to improve these results, and outside clinical trials, new approaches must consider long term toxicity, mainly the risk of secondary neoplasm and cardiopathy, which were responsible of the 22 % of deaths in our series.

Myeloma - Biology 2

0806

THE SDF-1 INHIBITING SPIEGELMER NOX-A12 DISRUPTS THE INTERACTION OF MULTIPLE MYELOMA CELLS WITH THE BONE MARROW MILIEU IN VIVO, LEADING TO ENHANCED SENSITIVITY TO BORTEZOMIB

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Background. SDF1/CXCR4 axis plays a major role in homing and trafficking of multiple myeloma (MM) cells to the bone marrow (BM). We hypothesized that de-adhesion of MM cells from the surrounding BM milieu through SDF-1 inhibition will enhance MM sensitivity to therapeutic agents. We therefore tested NOX-A12, a high affinity l-oligonucleotide binder (Spiegelmer) to SDF-1 in MM, looking at its ability to modulate MM cell tumor growth and MM cell homing to the BM *in vivo* and *in vitro*. **Aims. 1**) To determine the *in vivo* localization of MM cells and SDF-1 within the BM niches. 2) To evaluate the ability of NOX-A12 to induce BM de-adeshion of MM cells thus enhancing MM cell sensitivity to proteasome inhibition. **Methods.** BM co-localization of MM tumor cells (MM.1S-GFP+) with SDF-1 (AlexaFluor633-conjugated-anti-SDF-1-mAb) has been tested *in vivo* by immunoimaging, using *in vivo* confocal microscopy. Detection of mobilized MM-GFP+ cells *ex vivo* has been performed by flow cytometry. *In vivo* homing and *in vivo* tumor growth of MM cells (MM.1S-GFP+/luc+) were assessed by using *in vivo* confocal microscopy and *in vivo* bioluminescence imaging (BLI), in SCID mice treated with 1) vehicle; 2) NOX-A12; 3) bortezomib; 4) NOX-A12 followed by bortezomib. DNA synthesis and adhesion of MM cells in the context of NOX-A12 (50-100nM) treated primary MM BM stromal cells (BMSCs), in presence or absence of bortezomib (2.5-5nM), were tested by thymidine uptake and adhesion *in vitro* assay, respectively. Synergism was calculated by using CalcuSyn software (combination index: C.I. according to Chou-Talalay method). **Results.** We first showed that SDF-1 co-localizes in the same BM niches of growth of MM tumor cells *in vivo*. NOX-A12 induced a dose-dependent de-adhesion of MM cells from the BM stromal cells *in vitro*. These findings were corroborated and validated *in vivo*: NOX-A12 induced MM cell mobilization from the BM to the peripheral blood (PB) as shown *ex vivo*, by reduced percentage of MM cells in the BM and increased number of MM cells within the PB of mice treated with NOX-A12 vs. control (BM: 57% vs. 45%; PB: 2.7% vs. 15%). We next showed that NOX-A12-dependent de-adhesion of MM cells from BMSCs lead to enhanced MM cell sensitivity to bortezomib, as shown *in vitro*, where a synergistic effect between NOX-A12 (50-100nM) and bortezomib (2.5-5nM) was observed (C.I.: 0.570.76). These findings were validated *in vivo*: tumor burden detected by BLI was similar between NOX-A12- and control mice whereas bortezomib-treated mice showed significant reduction in tumor progression compared to the control (P<.05); importantly significant reduction of tumor burden in those mice treated with sequential administration of NOX-A12 followed by bortezomib was observed as compared to bortezomib alone treated mice (P <.05). Similarly, NOX-A12+bortezomib combination induced significant inhibition of MM cell homing *in vivo*, as shown by *in vivo* confocal microscopy, as compared to bortezomib used as single agent. **Conclusions.** Our data demonstrate that the SDF-1 inhibiting Spiegelmer NOX-A12 disrupts the interaction of MM cells with the BM milieu both *in vitro* and *in vivo*, thus resulting in enhanced sensitivity to bortezomib.

0807

GENE EXPRESSION OF OSTEOBLAST REGULATORS IN WHOLE BONE MARROW SAMPLES FROM MULTIPLE MYELOMA PATIENTS IN ASSOCIATION WITH OSTEOLYTIC BONE DISEASE

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Background. . The multiple myeloma (MM) plasma cell (PC) is dependent on the BM microenvironment. Most gene expression studies have been performed upon BM aspirates, which differ in cellular composition, or MACS isolated PCs. We used a new strategy with snap-frozen BM biopsies for gene expression of known osteoblast and osteoclast regulating genes to examine what is "actually going on in the BM of MM patients". **Aims**. Osteolytic bone disease (OBD) in MM is caused by a combination of inhibition of osteoblasts and hyperactivation of osteoclasts. We studied the expression of several genes related to the regulation of osteoblasts: Osteoblast (OB)-inhibitory factors in the HGF pathway: **HGF**, its receptor **cMET**, the co-receptor **Syndecan-1**, the partial cMET-

antagonist **Decorin** and **HGFActivator**, which are known to stimulate MM PC growth and inhibit OB. Also inhibitors of the OB-stimulating Wnt-pathway: **DKK1**, **SFRP-2**, **SFRP-3**, **Sclerostin** and **Wif-1** were measured, and the OB transcription factors **RunX2**, regulating early osteogenic differentiation, and **Osterix**, regulating OB maturation. **Methods.** During the diagnostic procedure we obtained an extra BM core biopsy which was snap-frozen. Biopsies were cut, homogenized and RNA was purified using the MagNa Pure Robot (Roche). cDNA was loaded for QPCR on 384-wells micro-fluidic cards (Applied Biosystems). Using 3 internal reference genes (ABL, GAPDH and GUS) the relative quantitative gene expression was calculated. OBD was evaluated using standard radiographs. All patients were untreated and did not receive bone-remodeling medicine. We examined 10 HV, 41 MGUS and 71 untreated MM patients, which according to radiographic findings were divided into NO/LOW and advanced OBD, i.e. OBD in ≥2 regions. **Results**. Gene expression of HGF, SDC1, DKK1, cMET and SFRP3 were significantly ($p<0.05$) associated with OBD with increasing gene-expression with increasing OBD, while Osterix were significantly associated with decreasing gene-expression with increasing OBD. DCN, SFRP2, SOST, Wif1, and RunX2 were not associated to OBD. HGFActivator was not expressed in any of our samples. When comparing only HV, MGUS and MM independent of OBD Sclerostin showed a tendency to be overexpressed in MM vs MGUs and HV ($p=0.08$). **Conclusions.** In our gene expression study reflecting the *in vivo* situation in MM, BM OB-regulators were significantly associated to OBD and/or disease status. The use of whole snap-frozen BM biopsies is a new method to evaluate gene expression in MM that makes it possible to investigate patients independent of degree of MM PC infiltration. Validation of some of the genes at protein levels will also be presented.

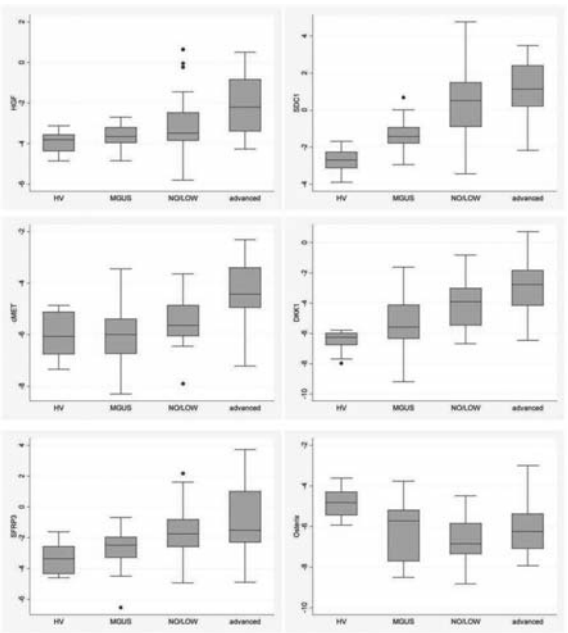

Figure 1. Delta Ct of significant genes.

0808

ASSOCIATION OF BASELINE CEREBLON (CRBN) EXPRESSION WITH CLINICAL RESPONSE, PROGNOSTIC PARAMETERS, AND β-CATENIN IN PATIENTS WITH MM TREATED WITH LENALIDOMIDE AND DEXAMETHASONE

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Background. Cereblon (CRBN) recently has been identified as a target for IMiD therapy in multiple myeloma (MM) and downregulation has been reported at the time point of resistance to lenalidomide (Zhu et al, Blood 2011). Accumulation of ß-catenin during treatment with lenalidomide might be another cause for drug resistance (Bjorklund et al, 2010; Chang et al, 2011). **Aims.** (1) To correlate baseline CRBN expression levels with clinical response in an enlarged patient cohort from 2 different centers, (2) to analyze the association of CRBN with clinical and prognostic parameters, and (3) to evaluate the association of CRBN with ß-catenin.

Table 1. Response quality and CRBN expression in 56 patients treated with lenalidomide an dexamethasone.

Quality of response	*N*	*Median CRBN expression*	*Range*
CR	7	3.78	0.80-9.90
nCR	2	4.82	3.74-5.89
VGPR	6	3.65	0.60-28.74
PR	25	2.09	0-6.70
MR	3	6.74	3.65-462.08
SD	10	0.78	0-2.37
PD	3	0.90	0.30-1.087

Figure 1A. Clinical response according to CRBN expression in patients treated with lenalidomide and dexamethasone (N=56).

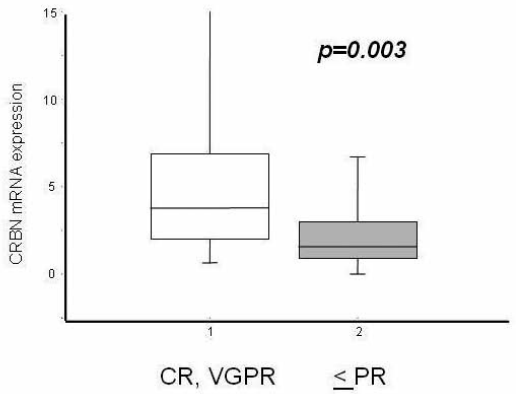
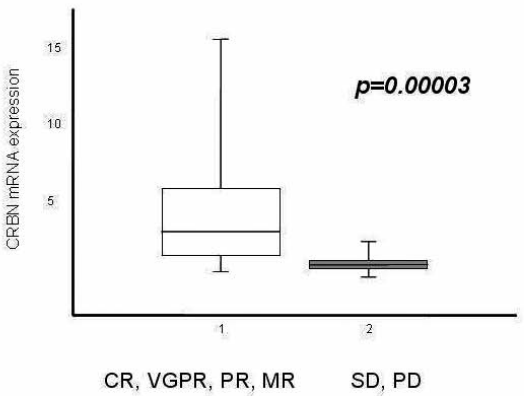


Figure 1B. Clinical response according to CRBN expression in patients treated with lenalidomide and dexamethasone (N=56).



Methods. We measured CRBN and ß-catenin expression by real time PCR in 56 bone marrow (BM) samples of well characterized MM patients uniformly treated with lenalidomide and dexamethasone (len/dex). The median age was 62 years; 20 patients had ISS-stage I, 22 stage II, and 24 stage III. Twenty-seven patients from Vienna (learning series) and 29 from Torino (validation series) were included. **Results.** Len/dex treatment resulted in CR in 7 (12%), nCR in 2 (4%), VGPR in 6 (11%), PR in 25 (45%), MR in 3 (5%), SD in 10 (18%), and PD in 3 (5%) patients. Median CRBN expression was 3.78 in patients with CR, 4.82 in patients with nCR, 3.65 in patients with VGPR, 2.08 in patients with PR, 6.74 in patients with MR, 0.78 in patients with SD, and 0.90 in patients with PD (**Table 1**) compared to normal BM (=1; N=8). Of note, only patients with SD or PD had a median CRBN expression lower than normal BM. CRBN expression correlated with the quality of response: ≥VGPR vs. PR-PD ($r=0.36$; $p=0.003$); ≥MR vs. SD, PD ($r=0.51$; $p=0.00003$) **(Figure 1)**. Among established prognostic parameters including ISS stage, beta-2-microglobulin, albumin, hemoglobin, and FISH defined cytogenetic aberrations, only beta-2-microglobulin correlat-

ed highly significant with CRBN (r=0.52; p=0.00004). A trend for lower CRBN expression in patients with a normal karyotype (N=11) was found (r=-0.28; p=0.04). Among all parameters, only the presence of del(13) (N=14), and t(4;14) abnormalities (N=9) were associated with an inferior response (p=0.04; p=0.01 respectively).The analysis of a possible correlation between CRBN and ß-catenin expression revealed a significant correlation between both genes (r=0.7; p=0.0000001). In 3 available patients, ß-catenin could be measured before start of lenalidomide therapy and after continuous exposure. ß-catenin was found to be upregulated, unchanged, and downregulated in each one of the 3 patients. **Conclusions.** Our data show a significant association between baseline CRBN expression and myeloma response in MM patients treated with len/dex in an enlarged patient cohort confirming previous data and the role of CRBN as an essential requirement for IMiD therapy. CRBN was found to significantly correlate with beta-2-microglobulin and was identified (among the parameters studied) as the strongest predictor for response. In contrary to our expectations, a close correlation between baseline CRBN and baseline beta-catenin was noted. Exposure to len/dex resulted in increased ß-catenin expression in only 1/3 patients. Thus, baseline elevated ß-catenin levels are unlikely accountable for lenalidomide resistance.

0809

CIRCULATING ANGIOGENIC CYTOKINES IN PATIENTS WITH SMOLDERING MYELOMA; IMPLICATIONS INTO DISEASE BIOLOGY

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Background. Angiogenesis is a critical step in the evolution of carcinogenesis in solid tumors and hematologic malignancies and is considered to be an early event in tumorigenesis. Multiple myeloma (MM) is a hematologic malignancy in which a preceding monoclonal gammopathy (MGUS) is considered a precursor. Asymptomatic/smoldering MM (SMM) is associated with a substantial risk of progression to MM and according to current recommendations these patients should be followed without therapy. **Aims.** In order to study the role and evolution of circulating angiogenesis related cytokines, we studied their levels in patients with MGUS, asymptomatic MM and symptomatic MM, before the initiation of first line therapy and their possible role as markers of early evolution. **Methods.** We measured serum levels of vascular endothelial growth factor (VEGF), angiogenin, angiopoietin (angp)-1 and -2, using standard ELISA methodology (R&D Systems, Minneapolis, MN, USA). The definition of MGUS, SMM and symptomatic MM was based on the IMWG criteria. All above cytokines were measured in 21 individuals with MGUS, in 174 newly diagnosed, untreated MM patients (31 with SMM) and in 44 age- and gender-matched healthy controls.We focused our analysis on patients with SMM. The median age was 63.5 years (range 40-83 years) and 55% were males. The median bone marrow infiltration in trephine biopsy was 20% (range: 12%-75%). Sixty-one per cent had IgG, 29% had IgA isotype while 3% had light chain only myeloma and 6% had biclonal myeloma. **Results.** The median (range) serum levels for VEGF were 406.5 pg/ml (186.3-797.6 pg/ml), for angp-1 were 31064 pg/ml (18220-50856 pg/ml), for angp-2 were 1434 pg/ml (486.1-4004.5 pg/ml), for angp-1 to angp-2 ratio were 20.8 (6.5-78.1), for angiogenin were 262732.6 pg/ml (138670-1003040 pg/ml) and for bFGF were 12.082 pg/ml (non-detected to 123.37 pg/ml). Patients with extensive bone marrow infiltration (60%) had significantly higher levels of ang-2 (p=0.017) and significantly lower angp-1/angp-2 ratio (p=0.004) compared to all others. Compared to healthy controls, patients with SMM had higher levels of angp-1 (p=0.05) and angp-2 (p=0.03) but their respective ratio was not significantly different (p=0.272). Serum levels of VEGF were significantly higher in SMM patients than in controls (mean 429 pg/ml *vs*. 196 pg/ml, p<0.001). Similarly serum levels of angiogenin were significantly higher in SMM (mean 304028 pg/ml *vs.* 190245 pg/ml, p<0.001). When patients with SMM compared to MGUS patients, there were no significant differences for any of the studied angiogenesis related cytokines. Compared to patients with symptomatic MM, patients with SMM had higher levels of angp-1 (p<0.001) and lower level of angp-2 (p<0.001) resulting in a significantly lower angp-1/angp-2 ratio, indicating a switch to increased vessel-formation activity, while the levels of VEGF were similar. **Conclusions.** The above results indicate that early in the myelomagenesis there is an angiogenic switch which is manifested by an increase in the levels of angiogenic cytokines that promote neovasculogenesis (such as VEGF, angp-2 and angiogenin) and a gradual suppression of cytokines that balance their effects (such as angp-1).

0810

LACK OF CORRELATION BETWEEN MCL-1 EXPRESSION AND SENSITIVITY TO ABT-737 DURING DISEASE PROGRESSION OF PLASMA CELL DISORDERS

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Background. Monoclonal plasma cells disorders (PCD) are clinically heterogeneous, including asymptomatic MGUS, smoldering multiple myeloma (SMM) and its progression to an overt disease represented by MM and plasma cell leukemia (PCL). Several molecular characteristics have been described in PCD, including the aberrant expression of the Bcl-2 family proteins, which prompt innovative therapeutic approaches based on the Bcl-2 targeted inhibition. However, neither potential differences in the expression of these protein members of the Bcl-2 family, nor differences in the activity of Bcl-2 inhibitors, have been associated with the clinical different status (SMM, MM and PCL), weakening the rationale of this novel therapeutic approach. **Aims**. We therefore examined the constitutive expression of the Bcl-2 family proteins (Bcl-2, Bcl-xL and Mcl-1) and the effects of the Bcl-2/Bcl-xL inhibitor, ABT-737 (kindly provided by Abbott Laboratories), on purified primary CD138+ cells from 11 SMM, 20 symptomatic MM (8 at diagnosis and 12 at relapse) and 4 PCL samples.METHODS: The cytotoxicity of ABT-737 on MM cells was established using the MTT assay. The drug concentration inducing 50% cell killing (IC-50) was calculated from the dose-response curve. Cell cycle inhibition and induction of apoptosis were analyzed by flow cytometry using the Acridine-Orange (AO) technique and by Annexin V binding assay. Cellular protein levels were evaluated by western blot analysis. **Results**. Our data indicate that the constitutive protein expression of Mcl-1 is significantly higher (p=0.059) in newly MM samples compared to SMM (mean ratio Mcl-1/GAPDH: 1.70±0.51 vs 0.95±0.27). Surprisingly MCL-1 expression decreases in the more aggressive PCD disorders (1.1±0.21 and 1.21±0.83 in relapsed MM and PCL, respectively). Bcl-2 and Bcl-xL did not differ between samples from these different groups of patients. A markedly ABT-737-induced pro-apoptotic activity was observed on purified CD138+ cells from primary samples. This effect was significantly (p=0.02) higher on CD138+ cells from SMM and on newly diagnosed MM compared to relapsed MM and PLC samples, which resulted progressively less sensitive to ABT-737. In particular, the pro-apoptotic activity of ABT-737 gradually decreased from SMM, newly diagnosed MM, relapsed MM and PCL, with a net apoptosis (sub-G0/1 peak) at 24 hours in the presence of 1000 nM ABT-737 of 55.9±15.2%, 49.3±21.3%, 37.6±10.6% and 33.3±17.9%, respectively. **Conclusions**. In summary, these data show that despite a Mcl-1 increase from SMM to MM, the sensitivity to ABT-737 is unchanged, supporting the recently reported hypothesis that the interaction between Mcl-1 and other pro-apoptotic members, such as Bim, is the major player in the response of MM cells to ABT-737. Moreover, the highest levels of sensitivity of SMM to ABT-737 associated with the lowest levels of Mcl-1 expression, prompt new investigational approaches for the management of patients with SMM

0811

TARGETING NAD+ SALVAGE PATHWAY INDUCES AUTOPHAGY IN MULTIPLE MYELOMA CELLS VIA MTORC1 AND EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK1/2) INHIBITION

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Background. Intracellular nicotinamide adenine nucleotide (NAD^+) is a coenzyme crucially involved in the regulation of several cellular processes. In mammals, NAD^+ stores are continuously replenished by a salvage pathway from Nicotinamide through the rate-limiting enzyme Nicotinamide phosphoribosyltransferase (Nampt). Indeed, tumor cells exhibit aberrant metabolic activity characterized by high levels of aerobic glycolysis and increased turnover of NAD^+ to support their rapid proliferation. In such scenario, promising results obtained in preclinical cancer models with Nampt inhibitors (such as FK866) suggest that Nampt activity represents an innovative therapeutic target for anticancer agents. **Methods.** a panel of 18 different MM cell lines, both sensitive and resistant to conventional and novel anti-myeloma drugs, and BM samples

from MM patients were used in the study. The antitumor effect of FK866 was investigated by Annexin-V/propidium iodide staining, thymidine incorporation, Western-blotting, lentivirus-mediated shRNAs and gene expression profiling analysis. Intracellular NAD^+ content was measured using a biochemical assay. Identification of autophagy was validated by 4 independent approaches: electron microscopy, proteolytic cleavage of endogenous LC3-I to LC3-II by Immunoblotting, formation of LC3 puncta pattern in GFP-LC3-transfected cells, as well as by gene expression profiling. Angiogenesis and osteoclastogenesis were measured in vitro using Matrigel capillary-like tube structure formation assay and osteoclast culture, respectively. In vivo study was performed using CB17-SCID mice xenografted subcutaneously with MM cells. **Results**. the chemical Nampt inhibitior FK866 triggered cytotoxicity in a panel of 18 MM cell lines and patient MM cells. Their viability was uniformly inhibited, with IC_{50} values at 96 hours ranging from 3-30nM. Additionally, Nampt inhibition killed, in a dose-dependent fashion, MM cells resistant to conventional and novel anti-MM therapies and overcome the protective effects of cytokines (IL-6, IGF-1), bone marrow stromal cells and primary osteoclasts. In contrast, FK866 treatment of PBMCs from 5 healthy volunteers did not affect cell viability. Nampt knockdown by RNAi confirmed its pivotal role in maintenance of both MM cell viability and intracellular NAD^+ stores. Interestingly, cytotoxicity of FK866 triggered autophagy but not apoptosis. A transcriptional-dependent (TFEB) and -independent (PI3K/mTORC1) activation of autophagy mediated FK866 MM cytotoxicity. Finally, FK866 synergized with conventional and novel anti-MM therapies and demonstrated significant anti-MM activity in a xenograft-murine MM model, associated with down regulation of ERK1/2 phosphorylation and proteolitic cleavage of LC3 in tumor cells. **Conclusions.** In conclusion, we identify for the first time a link between intracellular NAD^+ metabolism and autophagy in MM cells, providing the framework developing new targeted therapies in MM.

0812

DEPTOR EXPRESSION CHARACTERIZES GENETIC SUBGROUPS AND ASSOCIATES WITH MTOR SPECIFIC MICRORNAS IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is characterized by frequent and complex genomic abnormalities. In the majority of cases the PI3K/Akt/mTOR pathway is activated promoting growth, progression and resistance to therapy. This activation can be sustained by external factors, namely growth factors including insulin-like growth factor-1 (IGF-1) and Interleukin-6 (IL6). Recently, an mTOR interacting protein named *DEPTOR* was found to be highly expressed exclusively in MM (Peterson et al. *Cell* 2009). Importantly, knockdown of *DEPTOR* results in prevention of MM cell growth and apoptosis making it an attractive potential therapeutical target. **Aims.** Therefore, we aimed to determine if *DEPTOR* expression associates with clinically relevant subgroups. In addition, to investigate additional regulatory layers we determined key microRNAs (miRNAs) downstream of *DEPTOR* expression. **Methods.** *DEPTOR* expression was measured by quantitative real-time RT-PCR (TaqMan) using CD138 purified plasma cells from 175 patients with MM. *DEPTOR* copy numbers were measured and normalized to GUSB (internal control) copy numbers. In 38 patients miRNA expression levels were analyzed using Agilent mirRNA-Chips to measure miRNAs associated with *DEPTOR* expression. Additionally; all patients were characterized by a comprehensive set of FISH probes for the presence of recurring cytogenetic abnormalities. **Results.** *DEPTOR* expression was highly variable in the investigated MM samples (median: 0.33; range: 0.003 - 12.50). *DEPTOR* expression was significantly higher in patients presenting with translocations involving the immunoglobulin heavy-chain locus compared to patients with a hyperdiploid karyotype (p=0.0016). In particular, high *DEPTOR* expression was associated with the presence of a translocation t(14;16)(p<0.0001) and a deletion 13q14 (p=0.04), whereas low *DEPTOR* expression was associated with the presence of a gain at chromosomal band 9q34 (p=0.0006).Recently, activation of the mTOR pathway has been shown to be under the control of miRNAs, regulating tumor growth and differentiation. We detected twelve differentially expressed miRNAs (p<0.05), 8 miRNAs positively and 4 miRNAs negatively correlated with high *DEPTOR* expression. Of particular interest, miRNAs targeting the mTOR pathway (miR-99a, miR-193b, miR-365) or regulating IL6-Expression (miR-365) were found to be upregulated in high *DEPTOR* expressing patients, indicating a regulatory loop between mTOR activation and *DEPTOR* expression.**Summary.** High *DEPTOR* expression is associated with prognostic relevant genetic aberrations and specific miRNAs in MM, further dissecting the impact of *DEPTOR* expression on mTOR signaling.

0813

NATURAL KILLER CELL ACTIVATION, CYTOKINE PRODUCTION, AND CYTOTOXICITY IN HUMAN PBMC / MYELOMA CELL CO-CULTURES EXPOSED TO ELOTUZUMAB ALONE OR IN COMBINATION WITH LENALIDOMIDE

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Background. Elotuzumab (Elo) is a monoclonal IgG1 antibody targeting CS1, a cell surface glycoprotein highly expressed on >95% of myeloma cells. The primary mechanism of action of Elo is NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) of $CS1^+$ myeloma cells. Lenalidomide (Len) is an immunomodulatory agent reported to increase NK cell activity and may therefore provide additional therapeutic benefit in combination with Elo. The combination of Elo + Len synergistically enhanced anti-tumor activity in preclinical myeloma xenograft models. **Aims**. To investigate the mechanism of enhancing NK cell activation and myeloma cell killing with Elo in combination with Len. **Methods.** Human PBMC/OPM-2 myeloma cell co-cultures were treated for 24-72h with Elo (10-20 µg/mL), Len (1 µM), or Elo + Len. Activation markers and adhesion receptors were evaluated by flow cytometry. Cytokines were measured by Luminex and ELISpot assays. Cytotoxicity was assessed by absolute cell counting.

Treatment	IFN-γ (pg/mL) ave n=8	ICAM-1 on OPM-2 (MFI) n=4	CD25 on NK cells (MFI) n=8	IL-2 (pg/mL) (Luminex)		# IL-2 positive colonies (ELISpot) n=9	OPM-2 killing (%) n=4
				No blocking (control mAb) ave n=6	Anti-CD25 blocking mAb ave n=6		
*Control	48	427	155	21	-	30	1
**Lenalidomide	94	2040	131	50	-	71	15
Elotuzumab	65	3068	975	7.8	52	80	39
Elotuzumab + Lenalidomide	386	42,259	1546	14	134	191	67

*clgG1 (isotype); **plus clgG1.

Results. IFN-γ secretion increased significantly in Elo + Len treated cultures compared to Elo or Len alone. IFN-γ is known to elevate ICAM-1 expression and ICAM-1 surface expression on OPM-2 target cells increased synergistically with Elo + Len. Elo, Elo + Len, but not Len alone, increased the expression of CD25 (IL-2Rα) on NK cells. Len increased the level of soluble IL-2 but levels were decreased in the presence of Elo. The decrease in soluble IL-2 was likely due to increased consumption by CD25-expressing NK cells as blocking the uptake of IL-2 with anti-CD25 resulted in higher IL-2 levels than with Len alone. ELISpot assays confirmed that Elo + Len significantly increased the number of IL-2-producing cell colonies compared with cultures treated with Elo or Len alone. Elo alone induced NK-dependent myeloma cell killing and killing was significantly higher with the combination of Elo + Len. **Conclusions.** Elo alone activated NK cells and mediated the killing of myeloma cells in PBMC/OPM-2 co-cultures. The combination of Elo + Len synergistically increased IFN-γ secretion, ICAM-1 expression on myeloma cells, CD25 expression on NK cells, IL-2 production and consumption, and enhanced inhibition of myeloma cell growth.

0814

KDE AS AN ALTERNATIVE MOLECULAR TARGET FOR MINIMAL RESIDUAL DISEASE ASSESSMENT BY RQ-PCR IN PATIENTS WITH MULTIPLE MYELOMA

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Background. In multiple myeloma (MM) patients, the use of ASCT and novel agents is associated with high rates of complete response and thus, additional methods assessing higher degrees of disease eradication are required. Minimal residual disease (MRD) detection by immunoglobulin heavy-chain (IGH) real-time quantitative PCR (RQ-PCR) and multiparameter flow cytometry (MFC) has proved to be of prognostic value in MM patients, with the former showing higher sensitivity but lower applicability, in part due to the lack of a suitable target free of somatic hypermutation (SHM). *KDE* rearrangements occur in virtually all Ig-lambda B-cell malignancies and in 1/3 of Ig-kappa, are not affected by SHM and, as in ALL, could be used as PCR-targets. **Aims.** 1) To evaluate the incidence, gene segment usage and CDR3 composition of *IGK-KDE*

rearrangements in MM patients. 2) To test KDE as an alternative molecular target for MRD detection in patients with MM. **Methods.** A total of 96 untreated MM patients enrolled in GEM/PETHEMA protocols were included. The identification of the *IGK-KDE* rearrangements was performed according to the BIOMED-2 Concerted Action. IGH RQ-PCR was carried out using a germline reverse primer and a germline Taqman probe in combination with allele-specific oligonucleotides (ASO) as forward primers. Sensitivity and quantitative range were determined following EuroMRD recommendations (*Van der Velden, Leukemia 2007*). **Results.** Monoclonal *KDE* rearrangements were identified in 45% of the cases (29 monoallelic and 14 biallellic). Overall, 88% of cases (38/43) were successfully sequenced, *KDE* being equally frequently rearranged with *VK* and with intron-RSS. *VK* family usage was in line with normal B cells. Most cases (94%) were non-mutated (cut-off=2%). Median numbers of inserted and deleted nucleotides in the junctional region were one and five, respectively. Regarding the RQ-PCR analysis, sensitivity ≤5 x10^{-4} was reached in 44% of the ASO-primers designed to test *KDE* as a PCR target. **Conclusions.** *KDE* rearrangements can be found in approximately 50% of cases in MM, they can be quite easily sequenced and are essentially free of SHM. *KDE* rearrangements as alternative PCR targets for MRD assessment in MM improve the applicability of these studies in 9% of cases overall and in 20% of lambda cases. Due to the singular characteristics of *KDE* in MM frequent background. amplification could be observed, thus reducing the sensitivity of this marker as compared to ALL.noepuig@gmail.com

0815

QUANTITATIVE DETECTION OF FOUR CANCER-TESTIS ANTIGEN GENES IN MULTIPLE MYELOMA HAS CLINICAL VALUE

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Background. Cancer-testis (CT) antigen gene might promote the progression of multiple myeloma, and are considered as potential diagnostic and prognostic markers in myeloma. However, the expression levels and clinical implications of these genes are not fully understood in multiple myeloma. **Aims.** This study aimed to measure the expression levels of four CT antigen genes in Chinese MM patients and explore their clinical implications.

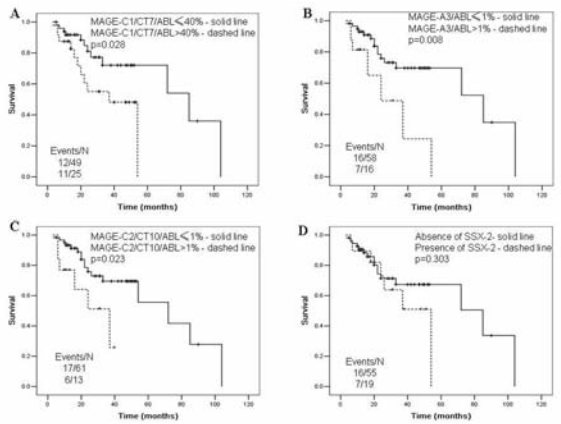

Figure 1. Overall survival (OS) analysis according to the qPCR expression levels of CT antigens: (A) MAGE-C1/CT7, (B) MAGE-A3, (C) MAGE-C2/CT10, (D) SSX-2

Methods. *Real-time quantitative polymerase chain reaction (qPCR) was used to quantify MAGE-C1/CT7, MAGE-A3, MAGE-C2/CT10 and SSX-2 mRNA expression in 238 bone marrow samples from 138 multiple myeloma patients.* The correlation between CT antigen expression and various clinical characteristics, and the clinical implications for molecular diagnosis, prognostic evaluation and treatment efficacy monitoring were determined. **Results.** *The qPCR method could sensitively quantify CT antigen expression. In the newly diagnosed, the positive expression rates were 90.0% for MAGE-C1/CT7, 84.3% for MAGE-A3, 84.3% for MAGE-C2/CT10 and 32.9% for SSX-2. MAGE-C1/CT7, MAGE-A3 and MAGE-C2/CT10 were frequently and persistently co-expressed in multiple myeloma patients. The expression levels and the number of co-expressed CT antigens correlated significantly with several clinical indicators, including the percentage of plasma cells infiltrating bone marrow as well as the clinical course of multiple myeloma and reduced overall survival.* **Conclusions.** *MAGE-C1/CT7, MAGE-A3 and MAGE-C2/CT10 expression leveles may provide potentially effective clinical indicators for auxiliary diagnosis, prognostic evaluation and monitoring of treatment efficacy in multiple myeloma.*

0816

QUANTITATIVE PHENOTYPING AND DISCRIMINANT ANALYSIS IMPROVE IDENTIFICATION IN MGUS DISORDERS

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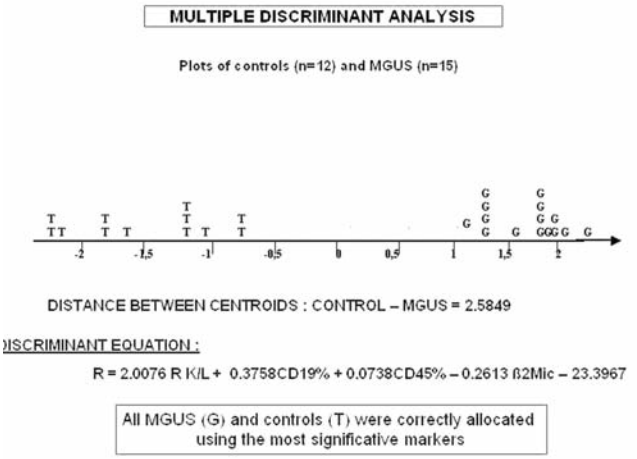
Background. Monoclonal gammopathy of undetermined significance (MGUS) is a common plasma cell dyscrasia with controversial phenotype definition. **Aims**. The purpose of this study was to identify antigens that could be used to differentiate MGUS, myeloma and normal plasma cells. **Methods.** Multivariate analysis classification of these three populations is investigated in 115 patients by an extended panel of surface markers. Diagnosis was based on clinical features, bone marrow cytology and immunophenotyping. Results were obtained on directly labeled CD38 and CD138 plasma cells by six-color flow cytometry. We examined percentages and antigen fluorescence intensities for CD27, CD19, CD45, CD20, CD117, CD200, FGF-R3, CD56, CD191, β7 Integrin, kappa/lambda intracytoplasmic chains and serum β2-microglobulin level. Efficiency of this panel was assessed by Chi-Square test. Principal components analysis and discrimant multivariate approach were used with the selected best markers for predictive classification.



Results. For CD19%, CD45%, CD117%, kappa/lambda chains and β2-microglobulin level, χ^2 values indicated very high probability of correct classification for MGUS and normal plasma cells (varing from 3.882 to 2.022 ; $10^{-4} \leq p \leq 10^{-2}$). MGUS and myeloma χ^2 values were more significant for CD19%, CD45%, CD27%, kappa/lambda chains and β2-microglobulin (varing from 3,882 to 3,019 ; $p \leq 10^{-4}$). Percentages of CD20, CD117, β7 Integrin and fluorescence intensity of CD27 and CD56 gave low χ^2 values (varing from 2,104 to 2,000 ; $10^{-4} \leq p \leq 10^{-3}$). It should be noted that the effectiveness of percentages was much more significant than fluorescence intensities for all markers. Principal component analysis of CD19%, CD45% and kappa/lambda chain ratio showed separated points in the plot representing patients with MGUS and normal plasma cells. This representation confirmed the high predictive potential of our panel. In order to find the optimal combination of the selected best markers, a stepwise probit discrimination was performed. 100% of MGUS and normal plasma cells were correctly classified with kappa/lambda chains, CD45, CD19 and β2-microglobulin. It was 88.89% with three parameters (% of kappa/lambda chains, CD45 and CD19) and only 77.78% with two parameters (% of kappa/lambda chains and CD19). If myeloma were added, efficiency decreased to 50.0% correctly identified patients.5. **Conclusions.** Therefore, it is reasoned that phenotypic analysis of plasma cells with kappa/lambda chains, CD45, CD19 and assessment of serum β2-microglobulin could detect malignant plasma cells in MGUS with moderate plasmacytosis. Finally, our study may prove the utility of multivariate analysis, which correctly differentiates normal plasma cells and MGUS.

0817

BENDAMUSTINE-INDUCED MYELOMA CELL APOPTOSIS DEPENDS ON REACTIVE OXYGEN SPECIES AND IS PARTLY P53 DEPENDENT

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Background. Multiple Myeloma (MM) patients carrying del(17p13), which overlap the TP53 locus, are considered to have a high-risk disease. Treatment with alkylators does not overcome this poor prognostic factor. Bendamustine, an alkylator/purine analogue hybrid used as chemotherapeutic agent, has demonstrated clinical efficacy in lymphoproliferative disorders and MM. Although bendamustine has been used for more than 30 years, its precise mechanism of action is still poorly understood and no prognostic markers has been reported for patients. **Aims.** The aims of this study were 1) to define the efficiency of bendamustine to induce cell death of myeloma cells, 2) to define whether p53 impacts this efficiency and 3) to characterize the pathways controlling cell death. **Methods.** The cytotoxicity of bendamustine was assessed in 22 MM cell lines (8 $TP53^{WT}$ and 14 $TP53^{Abnormal}$) by measuring cell death using flow cytometry. Molecular cell death pathways were analyzed by western blot. Stable p53 silenced cell lines were used to directly address the involvement of p53. **Results**. Bendamustine induced cell death in all cell lines although with different LD50 values ranging from less than 25 µg/ml up to 200 µg/ml. $TP53^{wt}$ cell lines were significantly more sensitive to bendamustine than $TP53^{Abn}$ cell lines (p=0.01). Moreover, cell death was significantly decreased in shp53 NCI-H929 compared with shCont NCI-H929 (p=0.019). In $TP53^{wt}$ cells, but not in $TP53^{Abn}$ cells, bendamustine increased expression of 3 p53 target genes, p21, Puma and Bax in correlation with cell death. In contrast, bendamustine increased Noxa expression in both $TP53^{wt}$ and $TP53^{Abn}$ cells. Apoptosis was associated with cleavages of caspases 3 and 9 but cell death was only partly prevented by the pan-caspase inhibitor z-VAD-fmk, suggesting the involvement of another mechanism. Indeed, cell death was strongly inhibited by a reactive oxygen species (ROS) scavenger, N-Acetyl-Cysteine, pointing out that production of ROS was essential. **Conclusions**. Our results show that bendamustine induced myeloma cell death through both p53 dependent and p53 independent mechanisms, in which ROS production plays an important role. Further investigations are required to define this exact process.

0818

DETERMINING OF MOLECULAR SIGNATURE FOR CENTROSOME ABNORMALITIES IN MULTIPLE MYELOMA

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Background. In multiple myeloma (MM), biologic complexity originates from complex oncogenic processes involving somatic acquisition of myriad mutations coupled with genetic variability within the host. This pathogenically determined molecular heterogeneity predetermines clinical heterogeneity. **Aims.** In this study, we performed gene expression profiling (GEP) focusing on centrosome related genes to determine molecular signature characteristic for centrosome abnormalities in MM patients. **Methods.** In total, 73 patients were evaluated for current study. The patient baseline characteristics were as follows: males/females 37/36, median age of 69.5 years (range, 46-85 years). Newly diagnosed (38/73) and relapsed (35/73) patients were included in this study; most of them had advanced stage of MM (DS II/III n =52; ISS II/III n=68). CD138+ cells were separated by MACS. All patients were evaluated by GEP using Affymetrix GeneChip Human Gene ST 1.0 array. In total, 111 genes were selected for clustering. All of these genes are involved in regulation of centrosome duplication and have known function in oncogenesis. Interphase FISH with cytoplasmic immunoglobulin light chain staining and qRT-PCR were performed on the same PC samples. **Results**. We have found one distinct pattern of 35 genes belonging to following ontologies: cell-cycle genes (*CDK1*, *CDK2*, *E2F*, *Plk*, *AURKA*, *NEK2*); kinetochore and microtubule attachment genes (*AURKB*, *BIRC5*, *CENP*); mitotic checkpoint genes (*BUB1, MUD2)*; DNA damage checkpoint genes presented by CA suppressors, integrity and reduplicate regulators (*RAD51, XRCC2, MSH2, CHEK1*) and just one structural gene TUBG1. Based on expression of these genes, patients can be stratified into three groups - 'High-', 'Mediate-' and 'Low-expressed'. The expression levels of genes selected from revealed pattern (*AURKA, AURKB, CCNB1, CCNB2, HMMR, PLK4, TACC3*, *CDK2*) were validated by qRT-PCR. PCR-based results clearly corresponded with GEP-based findings in mentioned above three subgroups of MM patients. We have found significant differences in relative quantification coefficient R of all 8 analyzed genes (*P*<0.05).There were no significant differences between revealed subgroups of MM patients regarding the presence/absence of common for MM cytogenetic aberrations. Patients in "Low expressed' group showed significantly higher level of hemoglobin (P=0.008) and thrombocytes (P=0.018), otherwise patients in "Mediate-"and "High expressed"group had significantly higher b2m (P=0.004), LDH (p=0.019) and plasma cell infiltration in bone marrow (P=0.042). Although statistical significance was not reached as a result of the small numbers of patients (P=0.098), however, in newly diagnosed patients significant difference between subgroups was observed (*P*=0.005). There was worse OS of patients in "High"and "Medium"subgroups than in patients in 'Low' subgroup. **Conclusions**. We defined a gene pattern, which was used as a molecular signature of centrosome abnormalities. Defined associations with integral clinical parameters and OS allow us to suggest the impact of revealed functional gene set in MM genesis. Our findings still need to be confirmed and validated on a larger external cohort of patients. **Funding**. This work was supported by grants: NT11154, NT12130, MSM0021622434 and by project GAP304/10/1395.

0819

EXPRESSION OF MCL1, GSTP1, AND CKS1B GENES IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

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Background. Monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) comprise heterogeneous disorders with incompletely understood molecular defects and variable clinical features. Molecular studies have identified genes differentially expressed in MM but some of them have been scarcely explored in MGUS patients. **Aims.** The aim of this study was to evaluate mRNA expression of *MCL1, GSTP1* and *CKS1B* genes in patient with MGUS and MM in order to analyze their participation in the progression of disease. **Methods.** Bone marrow samples from 90 patients: 60 with MM (26 males; mean age: 66.4 years; range: 30-86 years; Durie & Salmon stage: I: 21%, II: 17%, III: 62%) and 30 with MGUS (9 males; mean age: 70.6 years; range: 41-84 years) and, mononuclear cells from 11 normal controls, were analyzed. Patients were studied at diagnosis. All individuals gave informed consent and the study was approved by the local Ethics Committee. Real-Time Quantitative PCR was used to quantify gene expression. For statistical analysis, Mann-Whitney test and Kendal coefficient were used. **Results.** Analysis of mRNA expression showed differences among evaluated genes. *MCL1* had overexpression only in MM patients (24.69±11.05) with significant differences with respect to controls (4.13±1.14) (p<0.0001). *GSTP1* and *CKS1B* genes showed abnormal expression in both MM and MGUS patients. Overexpression of *GSTP1* gene was observed in 32% of MM and 33% of MGUS patients. Although MGUS mRNA levels (0.05±0.01) were lower than those of MM patients (0.12±0.05), no significant differences between them were observed. The analysis of *CKS1B* also showed abnormal expression in a similar percentage of MM and MGUS patients (60% and 50%, respectively). Comparison of mRNA values showed higher *CKS1B* expression in MM (0.017±0.006) with respect to MGUS patients (0.004±0.001) (p<0.04) and in both entities compared to controls (0.002±0.0004) (p<0.04). The analysis of clinical characteristics in MM patients showed a positive association between β2microglobuline and *MCL1* mRNA levels (p=0.015). Expression of *CKS1B* was positively correlated with the percentage of bone marrow infiltration (p=0.0012; r^2=0.1942) and M protein levels (p=0.0005; r^2=0.2415). *GSTP1* gene expression did not show association with clinical parameters. **Conclusions.** To our knowledge, this is the first evaluation of *GSTP1* mRNA expression in MGUS patients. Our data showed deregulation of *GSTP1* and *CKS1B* genes in this disorder. Significant differences in *CKS1B* expression between MM and MGUS and a positive association with bone marrow infiltration suggest a role for this gene in the progression from MGUS to MM.

0820

PROGNOSTIC IMPACT OF SERUM IMMUNOGLOBULIN HEAVY/LIGHT CHAINS RATIO IN PATIENTS WITH MULTIPLE MYELOMA IN COMPLETE REMISSION AFTER STEM-CELL TRANSPLANTATION

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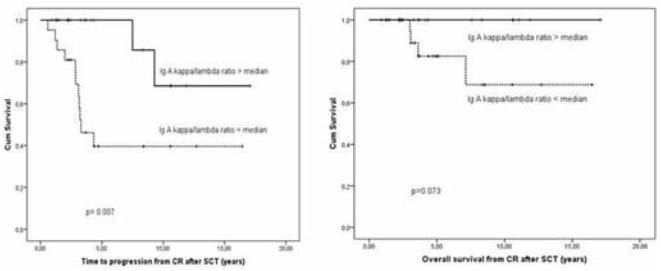
Background. Automatic assays for the measurement of serum free immunoglobulin chains have been available for a number of years and they have been useful for screening, diagnosis, prognosis and follow-up of monoclonal gammopathies. The recent development of antibodies against conjunction epitopes between the light and heavy chains has allowed for the first time to quantitate specific pairs of heavy/light chains (HLC) (IgGκ/IgGλ, IgAκ/IgAλ and IgMκ/IgMλ) in serum, which allows the determination of the quantity of tumoral immunoglobulin. In this context, the opportunity to determine HLC ratios provides a new tool to assess the response to treatment in MM. However, its prognostic contribution has not yet been determined. **Aims.** To establish the possible value of the serum HLC ratios in patients with MM in CR after stem-cell transplantation (SCT). **Methods.** Forty-two patients (19M/23F; median age 55 years) with MM in CR were studied. The patients were classified in two groups according to their previous treatments. The first one included patients who reached CR after melphalan-based autologous stem-cell transplantation (ASCT) (37, 88.1%), and the second one after allogeneic SCT (Allo-SCT) (5, 11.9%). Serum HLC measurement (Hevylite assay ®; kindly provided by Binding Site Ltd) was performed by immunonephelometry, and ratios were determined for each isotype (IgG, IgA, and IgM).



Results. On the overall series, an increased IgAκ/IgAλ ratio was associated with a longer PFS (p=0.007) and with a statistical trend for OS (p=0.073) (Figure 1). On the other hand, an increased IgMκ/IgMλ ratio was related to a longer PFS (p=0.006), with not significant impact on OS. Concerning the original isotype, neither PFS nor OS were modified in relation to the IgGκ/IgGλ ratio in those with an original monoclonal IgG isotype. However, a low IgAκ/IgAλ or a low IgMκ/IgMλ ratio was associated with a shorter PFS (p=0.075 and p=0.003, respectively). In the group of patients with an original monoclonal IgA isotype, a lower IgGκ/IgGλ ratio was also associated with shorter PFS (p=0.039) but no relationship with the IgA or IgM ratio was observed. **Conclusions.** We found that relative higher HLC ratio of the uninvolved immunoglobulin is predictor for a significantly longer PFS and even OS in patients with MM in CR. Since HLC ratios provide a measure of tumour immunoglobulinproduction plus immunoparesis rather than a marker of MRD, this parameter is more likely a surrogate marker of robust immune recovery. In MM, the achievement of a CR, with negative serum and urine IFEs, is the most significant prognostic factor. Furthermore, different CR categories are associated with different prognostic impact. In this context, the opportunity to determine HLC ratios provides a new tool to assess the response to treatment in MM. However, its prognostic contribution has not yet been determined.

0821

PERIOSTIN IS ELEVATED IN THE BONE MARROW MICROENVIRONMENT AND IN THE SERUM OF PATIENTS WITH MULTIPLE MYELOMA; CORRELATIONS WITH ADVANCE DISEASE CHARACTERISTICS

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Background. Periostin is a disulfide-linked cell adhesion protein that belongs to the fasciclin family and is mainly produced by stromal cells. Periostin is highly expressed in early osteoblastic cells and in the periosteum, where it acts as a regulator of bone remodeling. In very recent reports, periostin seems to stimulate the metastatic growth of cancer cells regulating the interactions between cancer stem cells and their metastatic niche. However, there is no information on the role of periostin in multiple myeloma (MM). **Aims.** The aim of the study was to evaluate the production of periostin by myeloma cells in vitro, and to measure periostin in the bone marrow plasma and the serum of a large number of MM patients in order to explore possible correlations with disease features. **Methods.** We measured periostin in the supernatants of six myeloma cell lines (LR5, MR-20, L363, U261,H929ST and JJN3), in the bone marrow plasma of 41 newly diagnosed patients with MM and in the serum of 198 MM patients (106M/92F, median age 73 years) at different phases of their disease: 33 patients had smoldering myeloma (SMM) at diagnosis, 105 symptomatic MM at diagnosis, 30 patients were at a plateau phase and 30 patients had relapsed MM after previous response to therapy. Circulating periostin was also measured in 23 patients with MGUS and in 30, gender- and age-matched, healthy controls. Evidence of bone involvement in all patients was documented using plain radiographs, while a series of bone markers was also evaluated. **Results.** The mean periostin level in the supernatants of the myeloma cell lines was 18.4 ng/ml (±SD: 6.14 ng/ml), with no differences between the different cell lines. The mean periostin levels of the bone marrow plasma of the 41 patients were 638 ng/ml (±268 ng/ml). The circulating periostin concentrations of symptomatic MM patients at diagnosis (mean±SD: 911±694 ng/ml) were increased compared to controls (537±190 ng/ml; p<0.001), to SMM patients at diagnosis (601±351 ng/ml; p=0.001) and to MGUS patients (633±271 ng/ml; p =0.002). Patients with MM at plateau phase had a borderline reduction of serum periostin concentrations (729±360 ng/ml) compared to symptomatic MM patients at diagnosis (p=0.05) but they continued to have increased levels compared to controls (p=0.013). Patients with relapsed MM had increased circulating periostin (938±847 ng/ml) compared to controls (p=0.016), MGUS (p=0.04) and SMM patients (p=0.04) but no significant difference compared to symptomatic patients at diagnosis. In symptomatic MM patients at diagnosis, serum periostin strongly correlated with beta2-microglobulin, LDH, ISS stage, bone resorption and extensive lytic bone disease. In the 41 patients with measurements in both bone marrow plasma and serum, there was a strong correlation between the two values (r=0.445, p=0.01). **Conclusions.** Our results suggest that there is slight periostin concentrations in the supernatants of myeloma cell lines, but there are high periostin levels in both bone marrow plasma and serum of newly diagnosed, symptomatic myeloma patients. Circulating periostin correlates with advanced disease features and thus it seems that periostin is also implicated in the biology of multiple myeloma.

0822

MONOCLONAL GAMMOPATHY: A GENE EXPRESSION STUDY

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Background. Eight families in Iceland have been identified with multiple cases of monoclonal gammopathies (MG), including monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM) and Waldenström's macroglobulinemia (WM). It has also been shown that there is a multi-step transformation from MGUS to MM. An *in vitro* poke-weed mitogen (PWM) stimulation assay identified 12 disease-free relatives of patients within four of the eight families as having hyper-responding (HR) B-cells producing immunoglobulin at > 2 SD above controls. **Aims.** We established an *in vitro* model of the germinal centre (GC) reaction and performed gene expression analysis on unstimulated samples and those collected after 14 days in GC culture to investigate if the GC model faithfully mimics *in vivo* GC. We also screened for differences between controls (related and unrelated) and the HR group. **Methods.** B cells were isolated from peripheral blood (PB) samples from each HR (n=10), unrelated control (n=10), and related (n=9). mRNA was extracted immediately from B cells at day 0 and from B cells after 14 days of stimulation in the *in vitro* GC culture and used to synthesize cDNA. Cy-3 labeled, samples were loaded onto oligonucleotide microarrays. Gene expression data were normalized and appropriate ANOVA models were applied to prepared data for analysis between study groups and stimulation days. Maximum likelihood and likelihood ratio tests and Benjamini and Hochberg correction were applied. Analysis methods included unsupervised hierarchical cluster analysis and ontology group testing using bioinformatic software programs recommended by the Gene Ontology database. **Results.** Day 0 and day 14 gene expression results clustered separately on a heatmap. Approximately one-third (14,277) of gene transcripts were significantly different after 14 days in the GC

culture ($p_{adjusted}$ <0.001). The genes grouped into multiple ontology categories as expected. The largest fold change (FC) differences (FC > 2.5) yielded numerous genes and ontology categories, such as cell proliferation, DNA packaging and repair, apoptosis and cell survival, which mimicked those described by a previous study as being specific to the GC compartment *in vivo*. No significant differences were detected between the HR study group and the control groups when adjusted for multiple testing, but data which were unadjusted for multiple testing showed different expression of 44 gene transcripts on day 14 (p_{raw} < 0.001). **Conclusions.**The gene expression results suggest that the culture model faithfully mimics an *in vivo* GC response. The HR group as a whole does not display a significantly different gene expression pattern. Further analysis is in progress.

0823

CHANGES IN PROTEIN PROFILES OF MULTIPLE MYELOMA CELLS IN RESPONSE TO BORTEZOMIB

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Background. Multiple Myeloma is a malignant B-cell neoplasm that is characterized by the accumulation of malignant plasma cells in the bone marrow. It is the second most common hematological disorder. Bortezomib is the first of a new class of anti-cancer agents known as proteasome inhibitor. Until now, the molecular mechanism of Bortezomib-induced apoptosis on Multiple Myeloma cells was explained but, to the best of our knowledge, there is not any study examining the changes in protein profiles of Bortezomib treated Multiple Myeloma cells in the literature. Protein profiling is a promising discovery tool to determine the protein-protein interactions, signaling pathways, and finding biomarkers for drug discovery. **Aims.** The objective of this study was to determine the changes in protein profiles of U-266 multiple myeloma cells in response to Bortezomib. **Methods.** At the first experiment part, the cytotoxic concentration of Bortezomib at 72 h was determined by XTT cell proliferation assay. Then, apoptosis was evaluated by measuring the changes in caspase-3 enzyme activity and loss of mitochondrial membrane potential (MMP). At the second part, total cellular proteins were isolated from U-266 cells (control group) and Bortezomib exposed U-266 cells and were visualized by running the proteins on Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Two-Dimensional Polyacrylamide Gel Electrophoresis (2D PAGE) respectively. After that, differentiated proteins (increased and/or decreased or appear and/or disappear) were recovered from gel to be applied for Matrix-Assisted Laser Desorption/Ionization-Time of Flight-Time of Flight (MALDI-TOF-TOF) mass spectrometry for protein identification. **Results.** IC50 value (drug concentration that inhibits cell proliferation 50%) of Bortezomib on U-266 cells was found as 17 nM. Then, apoptosis was evaluated by measuring the changes in caspase-3 enzyme activity and loss of mitochondrial membrane potential (MMP). Treatment of U266 cells for 72 h with 20 nM Bortezomib caused a significant loss of MMP (about 2.14-fold) and also resulted in 1.17-fold increase in caspase-3 activity. In order to elucidate and compare protein profiles of bortezomib treated and untreated groups, two individual gel images were imported into gel analysis software DECODON Delta2D Version 4.3. Using the software, these 2D PAGE gels were superposed and comparative detailed analysis was performed. The analysis demonstrated 37 differentially expressed protein spots, 5 proteins were newly formed, 10 proteins were lost, 12 proteins were up-regulated and 10 proteins were down-regulated in Bortezomib treated group as compared to untreated control group. Some of the identified proteins after mass spectrometric analysis are as follows: Apoptosis Regulatory Protein Siva (newly formed), Caspase recruitment domain-containing protein 14 (lost), Ras-related protein Rab-25 (upregulated), Nuclear factor NF-κB p105 subunit (downregulated). **Conclusions.** We continue to analyse the differentially expressed proteins in response to Bortezomib. As we understand the changes in protein profiles in response to Bortezomib, we can explain the side effects of Bortezomib while we can also suggest potential use of Bortezomib for the treatment of various diseases. Additionally, the data obtained by this study can be helpful for medical schools and drug designers and may also provide new treatments.

0824

INCREASED SERUM LEVELS OF MIP-1 ALPHA CORRELATE WITH BONE DISEASE AND ANGIOGENIC CYTOKINES IN MULTIPLE MYELOMA PATIENTS

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Background. Multiple myeloma (MM) is a malignancy with abnormal plasma cell growth within the bone marrow. Macrophage Inflammatory Protein-1 alpha (MIP-1 alpha), is a pro-inflammatory chemokine, crucial for immune responses towards infection and inflammation. It is produced by various cell types, particularly macrophages, dendritic cells and lymphocytes. It can activate human granulocytes, leading to acute neutrophilic inflammation, and also induce the synthesis and release of various pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha from fibroblasts and macrophages. MIP-1 alpha and its receptors, especially CCR1 and CCR5, play a major role in the pathogenesis of MM and MM-induced osteolytic bone disease. They can, directly and indirectly, stimulate tumor growth, being correlated with tumor burden, via upregulation of cell adhesion and cytokine secretion. In addition, they modulate the osteoclast/osteoblast balance, by inducing osteoclast differentiation and inhibiting osteoblast function. **Aims.** The aims of the present study was to determine if circulating MIP-1 alpha can be used as a marker of bone disease activity in patients with MM and if there is any relationship with indices of angiogenesis such as IL-18, basic fibroblast growth factor (b-FGF) and hepatocyte growth factor (HGF). **Methods.** We studied 60 newly diagnosed myeloma patients (34 male, 26 female, with mean age 58±13.4 years). According to Durie-Salmon staging system, 11 had stage I disease, 25 stage II and 24 stage III. Bone disease was graded according to skeletal involvement on x-ray (grade 0: no bone involvement, 1: one lytic lesion, 2: more than 1 lytic lesions, 3: bone fracture or vertebral collapse). Seventeen of them had grade 0, 11 grade 1, 11 grade 2 and 21 grade 3. Twenty-eight healthy persons were included in the study. MIP-1 alpha, IL-18, b-FGF and HGF serum levels were determined by ELISA, using commercially available kits. **Results.** All the above measured variables were significantly higher in MM patients compared to control group (p<0.001 in all cases). They were also higher with advancing disease stage (p<0.001 in all cases) and skeletal grade (p<0.04 for MIP-1 alpha and p<0.001 for the other cases). We also found significant correlations between MIP-1 alpha with IL-18 (r=0.596 p<0.001), b-FGF (r=0.509 p<0.001) and HGF (r=0.424 p<0.001).**Conclusions.** MIP-1 alpha seems to be a sensitive marker of bone disease activity in MM. The significant relationship with the measured angiogenic markers indicates the contribution of enhanced angiogenesis to the increased bone involvement seen in MM patients.

0825

RELATIONSHIP BETWEEN CIRCULATING BAFF LEVELS WITH PROLIFERATION MARKERS IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is an incurable plasma cell malignancy, characterized by an unlimited proliferation and accumulation of malignant plasma cells in the bone marrow. The interactions between the various mediators, secreted from MM cells, with the microenvironment, in the bone marrow, seem to be critical in the pathogenesis and growth of MM. B-cell activating factor (BAFF) is a cytokine that belongs to the tumor necrosis factor superfamily with a significant role in the maintenance of normal B-cells development and homeostasis. Soluble form is secreted by various cell types, such as monocytes, macrophages, dendritic cells, activated T-cells and neutrophils. BAFF is a potent B-cell growth factor and co-stimulator of immunoglobulun production. **Aims.** The aim of the study was to measure serum BAFF levels in newly diagnosed MM patients, to estimate whether they were influenced by the disease stage and status and to correlate them with other proliferation indices of the disease, such as serum interleukin-6 (IL-6), IL-10, IL-15 and bone marrow plasma cell infiltration and Ki-67 proliferation index (Ki-67 PI). **Methods.** We studied 54 newly diagnosed MM patients (25 male, 29 female with mean age 66.2±10.0 years). Nine had stage I disease, 23 stage II and 22 stage III. We also studied 32 of them in plateau phase, as well as 25 healthy controls. BAFF, IL-6, IL-10 and IL-15 serum levels were measured by ELISA, whereas plasma cell infiltration and Ki-67 PI were estimated in bone marrow specimens by immunohistochemistry. **Results.** All the above markers were higher in patients compared to

control group (p<0.001 in all cases). All of them were also increasing with advancing disease stage (p<0.001 in all cases) and were significantly decreased in the plateau phase (p<0.002 for IL-10, p<0.001 for the other cases). We found significant correlations between serum BAFF levels with IL-6 (r=0.711 p<0.0001), IL-10 (r=0.634 p<0.0001), IL-15 (r=0.642 p<0.0001) serum levels, bone marrow plasma cell infiltration (r=0.369 p<0.006) and Ki-67 PI (r=0.693 p<0.0001). **Conclusions.** Our data confirmed that BAFF is secreted in MM as soluble factor. Additionally we found that serum levels were higher with advancing disease stage. Finally, our results showed that there is a strong correlation with other known proliferation indices of the disease, such as IL-6, IL-10, IL-15, bone marrow infiltration and Ki-67 PI. This finding could raise concerns about its use as a target for therapeutic interventions in myeloma patients.

0826

GENOME WIDE SNP ANALYSIS REVEALS UNIPARENTAL DISOMY (UPD) AS A NEW MECHANISM IN GENE DYSFUNCTION IN WALDENSTROM'S MACROGLOBULINEMIA (WM)

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Background. SNP array was developed to combine the detection of CNV and LOH without copy number changes, also called UPD. Acquired UPD are not detectable by conventional cytogenetic or CGHa, and the regions with UPD might be important for the pathogenesis of cancer, and also lead to tumor suppressor gene inactivation or oncogene activation in cancer.Previous studies have demonstrated high frequency of chromosomal abnormalities in WM, a low grade B cell lymphoplasmacytic lymphoma type, using conventional cytogenetic, FISH and CGH analysis. We hypothesized that SNPa study would allow identification of novel genomic aberrations involved in WM, would permit a better understanding of WM pathogenesis, and favour the identification of novel targets for future drugs development. Our aim was to identify LOH/UPD involved in WM, and to characterize the clinical significance of these chromosomal defects. **Methods.** BM samples of 31 ptswith WM were analysed. DNA was extracted following B cells selection for tumoral cells. Genome-Wide Human SNP Array 6.0 (Affymetrix chips) was used. Paired samples (tumor/ normal T lymphocytes) were used as an intra-individual reference to identify germ line SNP. Size, position and location of genes were identified with UCSC Genome Browser HG18 assembly, LOH and CNA using genotyping console 3.02 software (Affymetrix) and Partek genomic suite. FISH analysis and conventional Karyotypes were available for all patients. **Results.** A total of 115 genetic aberrations (3.7 / patient) were observed, including 61 CNV (33 gains, 28 losses) in 58% of patients and 54 UPD (mean of 1.8 / genome). The area of CNV and UPD were widely distributed throughout the genome, and we have identified 12 recurrent regions of CNV and 7 of UPD. The UPD regions varied in size, from 0.8 to 114 mb, and the most frequent UPD were interstitial regions. We have identified 7 recurrent UPDs located in 17q, 4q, 3q and 13q. Several genes involved in cancer biology are located in these UPD regions. Only one patient had UPD detected on chromosome 6q, a hallmark of cytogenetic abnormalities observed in nearly half the WM, interestingly extending from 6q to the telomere. UPD regions did not cover known microRNA deregulated in WM. Although a higher frequency of more than 3 CNV was observed in patients with symptomatic WM, in our series, no difference in terms of UPD was observed in symptomatic and asymptomatic WM.In terestingly, large and telomeric UPD were only observed in symptomatic patients (14%). **Conclusions.** New cryptic clonal chromosomal lesions were observed and mapped in WM using genome wide SNP array in this study. We described a high frequency of UPD in WM, an important mechanism that might contribute to the inactivation of tumour suppressor genes and subsequently to the regulation of tumor progression in WM. This study revealed UPD as a new pathogenic mechanisms likely underlying WM pathogenesis, that might help deciphering the genome instability of patients with WM. The results of this study expand the view of the genomic complexity of WM with the identification of numerous and complex genetic aberration.

Myeloma - Clinical 2

0827

PANORAMA 2: A PHASE II STUDY OF PANOBINOSTAT IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED AND BORTEZOMIB-REFRACTORY MULTIPLE MYELOMA

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Background. Patients with multiple myeloma (MM) refractory to bortezomib and an immunomodulatory drug have limited treatment options and a poor prognosis. In a phase I study of patients with relapsed or relapsed/refractory MM treated with panobinostat + bortezomib, durable clinical responses were observed overall and in patients with bortezomib-refractory disease. **Aims.** To evaluate the efficacy of the combination of panobinostat + bortezomib + dexamethasone, we conducted PANORAMA 2, a phase II trial in relapsed and bortezomib-refractory patients with MM. **Methods.** PANORAMA 2 is a single-arm, phase II study of panobinostat (20 mg, oral) + bortezomib (1.3 mg/m^2, intravenous) + dexamethasone (20 mg, oral) in patients with relapsed and bortezomib-refractory MM. Treatment phase 1 (TP1) consists of eight 3-week cycles of panobinostat (thrice weekly) and bortezomib (twice weekly) during weeks 1 and 2, with oral dexamethasone administered on the days of and after bortezomib dosing. Patients demonstrating clinical benefit enter treatment phase 2 (TP2), which consists of four 6-week cycles of panobinostat (thrice weekly) and bortezomib (once weekly) during weeks 1, 2, 4, and 5, with dexamethasone on the days of and after bortezomib. The primary endpoint is overall response (≥ partial response [PR]) in TP1. All patients provided informed consent, and patients who discontinued are followed for survival. **Results.** Fifty-five patients with bortezomib-refractory MM were enrolled with 6 patients ongoing and 30 in follow-up. The median age was 61 years (range 41-88 years). Patients were heavily pretreated: the median number of prior regimens was 4 (range 2-11), and most patients (64%) received prior autologous stem cell transplant. Twenty-seven (49%) and 36 (65%) patients had bortezomib and dexamethasone in their most recent prior line of therapy, respectively. Eighteen patients achieved ≥ PR for an overall response rate of 33% (1 near complete response and 17 PR), and 13 patients achieved minor response (MR) for a clinical benefit rate of 56%. Three patients achieved a very good partial response (VGPR). Eighteen patients completed TP1 and entered TP2, and 2 have completed ≥ 12 cycles. Common adverse events (AEs) of any grade included fatigue (72%), thrombocytopenia (64%), diarrhea (64%), nausea (58%), dyspnea (40%), anemia (40%), decreased appetite (40%), dizziness (38%), constipation (34%), peripheral edema (32%), and peripheral neuropathy (30%). Common grade 3/4 AEs included thrombocytopenia (60%), fatigue (17%), diarrhea (17%), anemia (15%), pneumonia (15%), and neutropenia (13%). Only 1 patient (2%) experienced grade 3/4 peripheral neuropathy.**Conclusions.** The addition of panobinostat restored bortezomib sensitivity in heavily pretreated bortezomib-refractory MM patients. The combination of panobinostat, bortezomib and dexamethasone may provide an important treatment option for patients with bortezomib-refractory MM. The combination is generally well tolerated with several patients continuing on therapy long term.

0828

A RETROSPECTIVE ANALYSIS ON 397 SMOLDERING MULTIPLE MYELOMA (SMM) CASES: PROGNOSTIC FACTORS ASSOCIATED WITH PROGRESSION OF SMOLDERING MULTIPLE MYELOMA TO SYMPTOMATIC FORM

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Background. SMM is an asymptomatic plasma-cell proliferative disorder associated with a high risk of progression to symptomatic multiple myeloma (sy-MM). Patients with SMM meet the diagnostic criteria of MM [serum monoclonal component (MC) higher than 3 g/l and a proportion of bone marrow plasma cells (BMPC) ≥10%] in the absence of clinical manifestations. Prognostic factors for the progression and outcome of this disease are unclear. **Aims**. In this retrospective study, we have analyzed some predictors of development in sy-MM in SMM. Moreover, In some of these SMM patients, we also had the opportunity to compare the risk of progression predicted by the % of bone marrow plasma cell (BMPC) involvement observed in the bone marrow biopsies (BMB) versus that observed in bone marrow aspirates (BMA). **Methods.** For this study, 397 patients with SMM observed in 12 centers of the Multiple Myeloma GIMEMA Latium Working Group between 01/1980 and 07/2010 were analyzed. At progression to sy-MM, the severity of clinical presentation was graded according to the need of intensive supportive care. **Results**. After a median follow-up of 135 months, the cumulative incidence of progression (CIP) rates to sy-MM were 45%, 55% and 75% at 10, 15 and 20 years, respectively. Hb ≤12.5 gr/dl, MC ³ 2.5 gr/dl and BMPC ³60% were the only parameters negatively affecting the CIP. In particular, 7/397 (1.7%) patients with BMPC ³60% had a 5.6-fold increased risk of fast progression (within 2 years) to sy-MM. This progression occurred with severe clinical manifestations in 62% of cases. BMB was more sensitive for the detection of BMPC involvement, even though BMA was a more reliable indicator of a rapid progression to sy-MM. **Conclusions**. These data show that SMM patients with ³60% BMPC rate at diagnosis present a very rapid progressions to sy-MM characterized, in the majority of cases, by more severe clinical manifestations and suggest that these patients should probably be candidate to standard treatment soon at diagnosis. Moreover, BMA is more sensitive than BMB in the identification of these very high risk SMM patients.

0829

THROMBOSIS IS ASSOCIATED WITH INFERIOR SURVIVAL IN MULTIPLE MYELOMA

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Background. Patients with multiple myeloma are at an increased risk of venous thromboembolism (VTE) and arterial thrombosis, especially when treated with thalidomide and lenalidomide. In the general population, the occurrence of VTE is associated with a decreased survival. It remains unclear whether development of VTE and arterial thrombosis alters overall survival in patients with multiple myeloma. **Aims.** Aims of the study were to evaluate whether VTE and arterial thrombosis influence survival in multiple myeloma patients in a large population-based study. **Materials and Methods.** We assessed the impact of VTE and arterial thrombosis (using Swedish Patient-Registry) on survival in a population-based study on 9,399 multiple myeloma patients diagnosed in Sweden 1987 to 2005 using Cox proportional hazards models. We also performed landmark analyses that started follow-up time six months after diagnosis and assessed the impact of any thrombotic event during the first six months on overall survival. **Results.** We found multiple myeloma patients with (versus without) VTE to have a higher mortality at one-, five-, and ten-years of follow-up, with hazards ratio (HR)=2.9 (95% confidence interval (CI) 2.4-3.5), 1.6 (1.5-1.8), and 1.6 (1.4-1.7), respectively. The risk of dying was also increased among multiple myeloma patients with arterial thrombosis, HR=3.4 (3.0-3.8), 2.2 (2.0-2.3), and 2.1 (1.9-2.1) at one-, five-, and ten-years, respectively. Given that a multiple myeloma patient survived the first six months following diagnosis (landmark analyses), early VTE (within six months) was not associated with a higher risk of death at one year (HR=1.4; 95% CI 0.9-2.0), five years (1.1; 0.9-1.3), or ten years (1.0; 0.9-1.2) (Figure). In contrast, in landmark analysis, early arterial thrombosis was associated with a significantly higher risk of death at one year (1.5; 1.1-2.0), five years (1.4; 1.2-1.6), and ten years (1.4; 1.2-1.6). **Conclusions.** In this large population-based study including over 9,000 multiple myeloma patients, in contrast to prior smaller studies, we found the development of arterial and venous thrombosis to be associated with a significantly poorer survival. However, multiple myeloma patients with an early VTE that survived their first six months had similar survival as those without VTE. Our findings are important as they confirm that thrombosis in multiple myeloma is a serious complication, increasing morbidity and even mortality, also in the era of novel anti-tumor agents. The prevention of thrombosis in multiple myeloma is an important goal in the management of multiple myeloma.

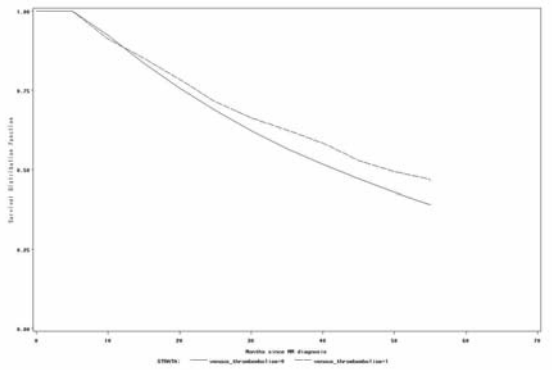

Figure 1. Survival among multiple myeloma patients with and without early venous thrombembolism (landmark analysis).

0830

DELETION 17P IN UNSELECTED NEWLY DIAGNOSED SYMPTOMATIC PATIENTS WITH MULTIPLE MYELOMA TREATED WITH NOVEL AGENTS IS ASSOCIATED WITH POOR OUTCOME DUE TO POOR POST RELAPSE SURVIVAL

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Background. Multiple myeloma (MM) is characterized by significant genetic heterogeneity. Specific cytogenetic features may be associated with poor outcome. Deletion of the short arm of the chromosome 17 (del17p), involving the p53 locus, has been associated with poor outcome. The frequency of this abnormality increases in more advanced phases of the disease. Recent clinical trials indicate that novel drugs may overcome the poor prognosis associated with some cytogenetic abnormalities, but it seems that neither bortezomib nor thalidomide or lenalidomide may overcome the deleterious effect of del17p. **Aims**. To assess the prognostic importance of del17p in unselected patients with MM, most of which received upfront novel agents. **Methods.** We analyzed 195 consecutive previously untreated patients, who were treated in a single center (Department of Clinical Therapeutics, University of Athens, Greece) with available data for del17p. Some of these patients were included in clinical trials; however, several patients who were ineligible because of poor performance status, significant renal impairment or comorbidities were also included in the analysis, thus, being more representative of the general myeloma population. Presence of del17p was assessed by standard FISH methodology. IMWG criteria were used for the assessment of response, progression-free (PFS) and

overall survival (OS). **Results.** del17p was present in 27(14%) patients at initial diagnosis. The baseline clinical characteristics of patients with and without del17p were not significantly different, except ISS-3 which was more frequent in patients with del17p (p=0.046). Primary therapy was based on novel agents (thalidomide, bortezomib or lenalidomide) in 86% of patients and was similar for patients with or without del17p (p=0.887); response was also similar (93% for those with del17p versus 84% for those without, p=0.140) as was the quality of responses (CR, VGPR & PR). Sixty-seven percent of patients with a del17p and 50% of those without del17p have relapsed or progressed after initial therapy. The median PFS for patients with and without del17p was 18.5 *vs*. 21 months, respectively (p=0.2); however, the median survival was significantly shorter for patients with del17p (29.5 versus 75 months, p<0.001). When we adjusted for other prognostic factors, then the presence of del17 was the stronger prognostic variable associated with poor survival (HR:3, 95%CI:1.7-5.3, p<0.001). Other factors associated with shorter survival included age >65 years (p=0.006) and elevated serum LDH ≥300 IU/L (p=0.012). On further analysis, we found that median survival after first disease relapse for patients with del17p was 8 months *vs*. 42 months for patients without del17p (p=0.002), despite the fact that in almost all patients novel agents were used as salvage treatment**. Conclusions.** del17p remains an independent prognostic factor associated with poor survival in unselected patients with newly diagnosed MM, even when novel agents are used as initial therapy. Patients with del17p have very poor outcome after relapse, even with the use of novel agents as salvage therapy. Patients with del17p should be considered for participation in clinical trials of novel agents as soon as they relapse, since currently available treatment options offer limited benefit in these high risk patients.

0831

AN UPDATE ON THE OVERALL SAFETY AND INCIDENCE OF SPM IN LENALIDOMIDE-, THALIDOMIDE-, AND BORTEZOMIB-TREATED PATIENTS WITHIN THE EUROPEAN POST-APPROVAL SAFETY STUDY (EU PASS) OF RELAPSED/REFRACTORY MM

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Background. The efficacy and tolerability of lenalidomide in patients with relapsed or refractory multiple myeloma (RRMM) has been demonstrated in 2 large, randomized, phase 3 studies (MM-009 and MM-010). Bortezomib and thalidomide are also proven to be effective for the treatment of RRMM. Post-approval observational studies provide additional information on the safety of novel medications in real-world clinical practice. **Aims.** To compare the incidence of adverse events (AEs) of special interest, such as neutropenia, thrombocytopenia, venous thromboembolism (VTE), peripheral neuropathy (PN), and second primary malignancies (SPM) in patients treated with lenalidomide and other antimyeloma therapies for RRMM in a real-world setting. **Methods.** In this observational, non-interventional, post-authorization safety study, patients with MM who had received at least one prior therapy were enrolled into the lenalidomide cohort (lenalidomide plus dexamethasone, the approved combination for the treatment of RRMM) or the background. cohort (all other treatments, including other novel agents) at the investigator's discretion. Thromboprophylaxis was allowed but not required. AEs were graded according to NCI-CTCAE (version 3) grading. Assessments for SPM were to be conducted up to 36 months after treatment discontinuation. **Results.** As of November 2011, 2,717 patients across 265 institutions, in 17 European countries were enrolled. Of those, 1,857 received lenalidomide, 670 received bortezomib, 101 received thalidomide, and 89 received other therapies or had missing data at time of analysis. Ninety-seven patients from the background. cohort crossed over following the physician's decision to initiate lenalidomide treatment. Median follow-up for all groups was 24 weeks (range, 0.6-157.3). Median age was 69 years (range, 29-95) and 54% were male. Most patients (66%) had good performance status (ECOG score 0-1); 17% had an ECOG score of 2-4. Median number of prior therapies was 2 (range, 0-6): 52% had 2 prior therapies and 22% had ≥3 prior therapies. Baseline characteristics were similar between groups. Overall, 1,189 (45%) had an AE of grade 3-4. Grade 3-4 neutropenia occurred in 19%, 7%, and 15% of patients in the lenalidomide, bortezomib, and thalidomide groups, respectively. Grade 3-4 thrombocytopenia developed in 16%, 14%, and 9% of patients, respectively. The overall incidence of peripheral neuropathy was 27% (4% grade 3-4) in the bortezomib group compared with 7% (1%) in the lenalidomide and 13% (1%) thalidomide groups. Grade 3-4 VTE developed in 2% of patients in the lenalidomide group and none in the other two treatment groups. The overall treatment discontinuation rate was 63%, 79%, and 78% in the lenalidomide, bortezomib, and thalidomide groups, respectively, with a similar discontinuation rate due to AEs in each group. The incidence of SPM was ≤1% overall (Table). Incidence of death during the study was similar with all treatments (lenalidomide 6%, bortezomib 5%, and thalidomide 5%). **Conclusions.** Lenalidomide has been shown to improve disease outcomes in RRMM patients. AEs, including SPM, were similar with all treatments except for higher rates of neutropenia and lower rates of PN with lenalidomide, compared with patients receiving bortezomib or thalidomide.

Table. Summary of SPM (*indicates same patient).

SPM	Len (n=1,875)	Bort (n=670)	Thal (n=101)	Total (N=2,628)
Invasive SPM, n (%)	8 (0.4)	4 (0.6)	1 (1.0)	13 (0.5)
Hematologic	2 (0.1) MDS 1pt, Leukemia 1pt	1 (0.1) AML 1pt	0	3 (0.1)
Solid tumor	6 (0.3) Colorectal cancer 4pts, Bladder cancer 1pt, Malignant melanoma 1pt*	3 (0.4) Colorectal cancer 1pt, Pancreatic cancer 1pt, Renal cancer 1pt	1 (1.0) Colon cancer 1pt	10 (0.4)
Non-invasive SPM, n (%)	4 (0.2) Basal cell carcinoma 2pts, Fibrous histiocytoma 1pt, Squamous cell carcinoma 1 pt*	0	0	4 (0.2)
Median time to invasive SPM, wks				
	33.1	19.3	62.1	–

0832

ALLOGENEIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA - A LONG-TERM FOLLOW-UP

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The treatment of multiple myeloma is still unsatisfactory. Although in recent years survival improvement was seen, disease progression just seems to be deferred. Even after high dose chemotherapy and autologous stem cell transplantation (ASCT), most patients relapse. Treatment options fo those patients are, despite promising newer drug, still limited. In contrast, allogeneic human stem cell transplantation (HSCT) bears the potential of definite cure, however, being hampered by inacceptable high Treatment Related Mortality (TRM) rates. Reduced intensity conditioning (RIC) regimens lead to a drop in TRM, but this benefit is offset by higher relapse rates. Hence, in three recently published prospective studies no consistent superiority in Overall Survival (OS)/Progression Free Survival (PFS) was seen for RIC HSCT when compared to ASCT. We provide the result of a retrospective, long-term follow-up (median 6,75 years), single center analysis of 78 patients receiving allo-HSCT for multiple myeloma from 1994 to 2009. Median age was 49 years, 25 Patiens had received more than one prior ASCT, 32 patiens had stable disease (SD) (n=5) or even progressive disease (PD) (n=27) at time of HSCT. Matched related donors were available on only 30 cases. Preparative regimen consisted of either Melphalan (once 140 mg/m^2 or twice 100 mg/m^2), TBI (8-12 Gy) based or reduced intensity regimen (TBI </=4 Gy, n=26) in combination with Cyclophosphamide, Fludarabin and ATG in virtually all cases. After HSCT a complete remission (CR) or very good partial remission (VGPR) was reached in 87% of all evaluable patients (five patients died prior to staging). The 2-year Non-Relapse Mortality (NRM) was 19,3% (total 24,4%). Chronic GvHD was seen in ca. 25% of patients. Risk factors being associated with diminished PFS and OS (given data) were >1 prior ASCT (OS:HR=2,80, p=0,00;PFS:HR=2,76;

p=0,00), allo-HSCT more than 10 months after last ASCT (OS:HR=2,09, p=0,012;PFS:HR=2,47, p=0,001), no partial remission (PR) at time of HSCT (OS:HR=2,41, p=0,002;PFS:HR=2,31, p=0,002), application of a 'reduced intensity conditioning' (OS:HR=2,11, p=0,009;PFS:HR=1,93, p=0,015) and a lower CD 34/MNC count (OS:HR=1,89, p=0,047;PFS:HR=1,82, p=0,040) in univariate analysis. In multivariate analysis >1 prior ASCT (OS:HR=2,81, p=0,001;PFS:HR=2,89, p=0,000) and 'RIC' (OS:HR=2,00, p=0,022; PFS:HR=2,09, p= 0,010) could be confirmed as independent risk factors. Patients reaching at least a PR prior to HSCT and lacking the latter two risk factor reached an OS of 60% and PFS of 50% with a follow-up till 17,5 years. Summarizing the results, we established >1 prior ASCT as an independent risk factor for subsequent HSCT, contradictory to the use of double ASCT as standard therapy, especially in younger patients. Furthermore we saw moderate TRM rates after 'myeloablative' HSCT, resulting in a significant better outcome compared to the RIC regimen. This observations raise the question if early and myelablative HSCT might be the key to cure multiple myeloma.

0833

OUTCOME OF PATIENTS WITH MULTIPLE MYELOMA FOLLOWING FIRST RELAPSE, AN IMWG STUDY

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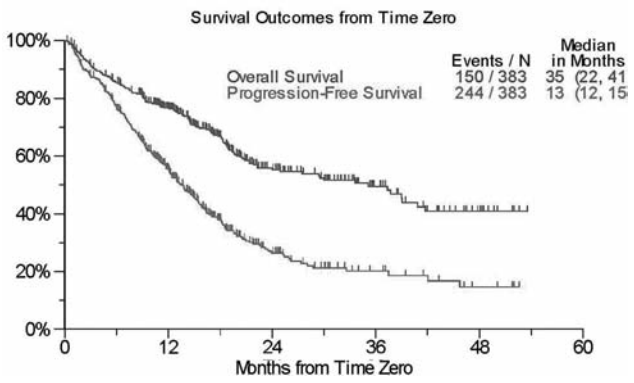
Background. Multiple myeloma remains incurable with current approaches and the disease invariably relapses after responding to initial therapy. There is no uniform approach to the management of relapsed disease and is dictated by the response to initial therapy, types of initial therapy and availability of new drugs. The outcomes associated with current approaches in this group of patients have not been systematically examined. **Patients and Methods.** We enrolled 383 patients with myeloma who had the first relapse of their disease between Jan 1, 2007 and June 30, 2010. Of the 383 patients, 26 were from North America, 220 were from Europe, 106 were from Asia, and 31 were from South America. Clinical data was collected using detailed case report forms for uniformity. Time Zero (T_0)was defined as the date of the first anti-myeloma treatment after first relapse. The Institutional Review Board at the respective institutions approved the study. **Results.** Across the study, 61% were male and 49% were over 65 years at T_0. ISS stage distribution at diagnosis included 26, 40 and 33% of patients in stages 1, 2 and 3, respectively. A variety of regimens were used at first relapse; bortezomib containing regimens were used most commonly (54%), followed by lenalidomide (25%) and cyclophosphamide (21%) containing regimens. The overall response rate (>=PR) to the first regimen after relapse was 58% including 14% with a complete response. The response rates were higher for the US and European centers (62 and 64% respectively). In a multivariate analysis, ISS stage 3, ISS stage 3 at relapse and Asian center were predictive of a lower response rate. There was a progressive decrease in the response rates with successive regimens; 45%, 30% and 15% for regimens 2, 3 and 4 respectively. Of the 109 patients considered eligible for stem cell transplant, 39% received an SCT as salvage therapy. Lenalidomide use was considerably lower in the Asian cohort, while bortezomib use was comparable across the regions. The median progression free survival (PFS) from T_0 was 13 mos and overall survival (OS) was 35 mos, for the entire cohort. In a univariate analysis, ISS stage 3, presence of cytogenetic abnormalities, history of plasma cell leukemia or extramedullary disease, bone marrow PC% > 33% and presence of renal insufficiency were all associated with a shorter PFS as well as shorter OS (P<=0.01). In a multivariate analysis including the maximum number of variables, ISS stage 3 and presence of EMD were most associated with short OS. **Conclusions**: Median progression free survival for current second line regimens, which typically contain bortezomib or lenalidomide in combinations appear to be around 1 year with an OS from first relapse of about 3 years. The OS from first relapse is nearly double that seen in a cohort of patients in first relapse prior to introduction of new drugs. Clear-cut regional differences can be seen in terms of patterns of drug use and health care resource utilization and likely reflect drug availability and healthcare costs.



0834

CONTINUOUS LENALIDOMIDE TREATMENT FOR TRANSPLANT-INELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA: UPDATE ON PATIENTS AGED 65-75 YEARS ENROLLED IN MM-015

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Background. MM-015 is a pivotal phase 3 randomized, double-blind, placebo-controlled trial designed to compare melphalan-prednisone-lenalidomide (MPR) induction followed by lenalidomide maintenance (MPR-R) with fixed cycle MPR and melphalan-prednisone (MP) induction in transplant-ineligible newly diagnosed multiple myeloma (NDMM) patients. Interim results demonstrated unprecedented progression-free survival (PFS) improvement (Palumbo A, et al. *Blood* 2011). **Aims.** Examine efficacy and safety in patients 65-75 years as a protocol-specified age group. **Methods.** Dosing and schedule have been described. Efficacy and PFS data are provided through the last adjudicated assessment (median follow-up of 27 months). The median follow-up for safety and overall survival (OS) is 41 months. **Results.** A total of 152, 153, and 154 patients were randomized to MPR-R, MPR, and MP, respectively. Median PFS was significantly improved with MPR-R (31 months) vs. MPR (14 months; *P*<.001) and MP (13 months; *P*<.001). A landmark analysis calculating PFS from the time of maintenance entry demonstrated that lenalidomide maintenance significantly prolonged median PFS (26 months from maintenance entry) vs. placebo (7 months) (HR=0.34; *P*<.001). Lenalidomide maintenance extended PFS independent of induction response (partial response vs. very good partial response or better), ISS stage (I/II vs. III) and age (65-75 years vs. >75 years). Patients aged 65-75 years were evenly distributed: 76% MPR-R patients (116/152), 76% MPR (116/153), and 75% MP (116/154). In these, MPR-R significantly prolonged median PFS vs. MPR and MP (Figure). Importantly, MPR induction alone improved PFS vs. MP (Figure). Lenalidomide maintenance reduced the progression rate by 65% (*P*<.001). PFS improvements were not-

ed in all patient subgroups (renal function, induction response, and ISS stage), and an OS trend has been observed (Figure). MPR induction had a manageable safety profile, allowing 67% of patients to reach maintenance. The most frequent adverse events (AEs) were hematologic. For patients aged 65-75 years, grade 4 neutropenia occurred in 31% (MPR) and 7% (MP); grade 4 thrombocytopenia was reported in 11% (MPR) and 4% (MP). Grade 4 febrile neutropenia was not reported. The most common grade 3/4 non-hematologic AE was infection (8% vs. 6%). Discontinuation from MPR and MP induction for AEs occurred in 12% and 4%, respectively. During lenalidomide maintenance, the most common AEs were grade 3/4 infections and bone pain (5% each). Eight percent discontinued lenalidomide maintenance due to AEs. Secondary primary malignancy (SPM) were uncommon corresponding to low incidence rates per 100 patient-years of 3.04, 2.57, and 0.98, respectively, which represent a total of 12 (MPR-R), 10 (MPR), and 4 (MP) SPM. Similarly, the risk of progression/death was higher than the SPM risk. **Conclusions.** Continuous lenalidomide treatment with MPR-R significantly extended PFS vs. MP and MPR. MPR induction significantly extended PFS vs. MP in patients aged 65-75 years. The manageable safety profile of MPR induction allowed 67% of patients to reach maintenance. Lenalidomide maintenance extended PFS in all patient subgroups analyzed. SPM were uncommon, although imbalanced across treatments. Overall, the risk of progression/death clearly outweighs the SPM risk. MPR-R should be considered a standard of care in transplant-ineligible NDMM patients aged 65-75 years.

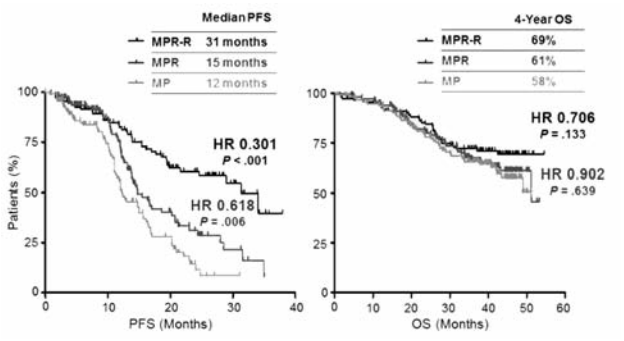

Figure 1. PFS and OS for Patients Aged 65-75 Years.

0835

BENDAMUSTINE, BORTEZOMIB AND DEXAMETHASONE (BVD) IN ELDERLY PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA : THE INTERGROUPE FRANCOPHONE DU MYELOME (IFM) 2009-01 PROTOCOL

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Bortezomib (V) plus Dexamethasone (D) is a treatment of choice of relapsed/refractory multiple myeloma. In small series, the addition of an alkylator was beneficial. Bendamustine (B) showed a high activity in advanced MM. The IFM 2009-01 trial evaluates the combination of B, V and D in elderly pts with MM progressive on or after 1st line therapy. We conducted a phase 2 trial combining B 70 mg/m^2 D1-8, V 1.3 mg/m^2 D1-8-15-22 and D 20 mg D1-8-15-22 every 28 days. 4 cycles were administered. In responders (PR or better), 2 additional cycles were provided followed by a maintenance phase with 6 cycles given every 2 months. Inclusion criteria were progression on or after 1 prior line of therapy, measurable disease, PS ECOG <3, ANC > 1.5x10^9/l, platelets > 100x10^9/l, creatinine < 250 mcmol/l, AST and ALT < 3xULN, provision of informed consent. Pts with prior exposure to bortezomib were excluded. Response was evaluated according to IMWG criteria. Primary end point was response at end of cycle 4, secondary objectives overall response rate (ORR), progression-free survival (PFS), overall survival (OS) and toxicity. The present analysis was restricted to the first 4 cycles. From 03/2010 to 07/2011, 73 pts were included, median age 75.8 years (range 66-86). Median time from diagnosis to inclusion was 29 months. All pts received only 1 prior therapy: MP in 12, MP-Thalidomide in 44, Lenalidomide-Dexamethasone (LD) in 14, other in 3. 42 pts (57.5%) were responders at end of cycle 4 [CR: 8 (10.9%), VGPR: 9 (12.3%), PR: 25 (34.2%), SD: 10 (13.6%), progression: 11 (15%), early discontinuation: 10 (13.6%)]. 6pts/10 were in PR and 1pt/10 in VGPR at time of discontinuation. ORR was 67.1% (49/73 pts). 11 pts died (MM: 6, sepsis: 4, renal failure: 1). Follow-up PFS and OS were 77.2% and 85%, respectively. Adverse events grade 3-4 were neutropenia: 16 pts, thrombocytopenia: 7 pts, sepsis: 12 pts, gastro-intestinal: 8 pts, anaphylaxis: 1 pt. 2 pts had DVT. Peripheral neuropathy grade>1 occurred in 9 pts, all grade 2. Treatment was stopped in 20 pts (lack of efficacy: 11, toxicity: 9). These results compare favorably with those achieved with VD or LD. The triplet BVD combination is very effective and tolerable in elderly pts with MM in 1st progression.

0836

RESPONSE RATES TO SINGLE-AGENT CARFILZOMIB IN PATIENTS DOUBLE REFRACTORY OR INTOLERANT TO BORTEZOMIB AND IMMUNOMODULATORS IN TRIAL PX-171-003-A1

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Background. Patients with relapsed and refractory multiple myeloma (MM) (1) whose disease is refractory to bortezomib or are intolerant of bortezomib and (2) whose disease is refractory to at least 1 immunomodulatory drug (ie, thalidomide or lenalidomide) or are intolerant of at least 1 immunomodulatory drug (ie, "double-refractory/intolerant patients"), have few therapeutic options and a poor prognosis. Carfilzomib, a next-generation proteasome inhibitor, has shown durable single-agent activity in clinical studies including 003-A1, an open-label, single-arm phase 2b trial in relapsed and refractory MM. **Aims**. The present analysis from study 003-A1 describes clinical activity of carfilzomib in double-refractory/intolerant patients and in other groups of clinical interest, including patients with disease refractory to all 5 approved classes of anti-MM treatments (alkylating agents, anthracyclines, corticosteroids, immunomodulatory drugs, and proteasome inhibitors) in clinical use ("refractory to all approved treatments"). **Methods.** Responses in double-refractory/intolerant patients from 003-A1 were analyzed, as were responses in patients with disease refractory to all 5 approved classes of anti-MM treatments. Carfilzomib was given on days 1, 2, 8, 9, 15, and 16 of each 28-day cycle (C), (20 mg/m^2 in C1; 27 mg/m^2 in C2-12). The primary endpoint was overall response rate (ORR; ≥ partial response [PR]). Secondary endpoints included clinical benefit response rate (≥ minimal response), duration of response (DOR), overall survival, and safety. **Results**. The ORR for all patients (N=266) was 22.9% with a median DOR of 7.8 months. In the 228 (86%) double-refractory/intolerant patients the ORR was 20.6% with a median DOR of 7.4 months. **Conclusions**. Patients with double-refractory/intolerant MM or disease refractory to all 5 approved classes of anti-MM treatment achieved clinically meaningful, durable responses with single-agent carfilzomib. The response rates across groups of clinical interest were generally consistent with the results for the entire study population. These results are notable for a next-generation proteasome inhibitor and

demonstrate the activity of single-agent carfilzomib in patients with advanced stage MM and its potential for meeting the unmet medical need in these patients.

0837

CLINICAL PROFILE OF MULTIPLE MYELOMA IN ASIA - AN ASIAN MYELOMA NETWORK (AMN) STUDY

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Background. The incidence of multiple myeloma (MM) is known to be variable according to ethnicity. However the difference of clinical characteristics between ethnic groups is not well-defined. In Asian countries the incidence of MM has been lower compared with Western countries. However, there are growing evidences that MM is increasing very rapidly in this region. Until now, only few data of Asian patients has been reported. Asian myeloma network (AMN) decided to analyze the first multinational project to explore clinical characteristics of Asian MM patients and clinical practice performed in Asian countries. **Aims.** We analysed the clinical characterics of Asian myeloma to characerize the differences with western data.

Table 1 Summary of clinical characteristics of AMN project

		n	%	median OS	p value
Total		2969	100.0	54.0	
Age	62 (19-105)				
	<65	1726	58.1	70.0	<0.001
	≥65	1243	41.9	39.0	
Gender	Male	1609	54.2	54.0	0.316
	Female	1360	45.8	52.0	
Bone lesion	yes	1140	48.6	54.0	<0.001
	no	1203	51.4	48.0	
Hemoglobin	<10.0 g/dl	1713	58.3	46.000	0.002
	≥10.0 g/dl	1127	38.7	66.000	
Platelet	<70 X 10 /l	522	18.8	32.000	<0.001
	≥70 X 10 /l	2288	81.4	59.200	
Extramedullary plasmacytoma	Absence	2307	86.5	52.0	0.597
	Presence	361	13.5	52.0	
Plasma cells in BM	<30%	1073	40.4	77.0	<0.001
	30-70%	1072	40.4	46.0	
	>70%	511	19.2	44.0	
Serum calcium	<10.5 mg/dl	2250	82.4	56	<0.001
	≥10.5 mg/dl	448	16.6	39	
Serum albumin	<3.5 g/dl	1144	56.3	50.000	<0.001
	≥3.5 g/dl	888	43.7	67.000	
Serum creatinine	<2.0 mg/dl	2123	76	60	<0.001
	≥2.0 mg/dl	679	24	27	
Serum beta 2-microglobulin	<3.5 mg/l	900	39.9	72.000	<0.001
	3.5-5.5 mg/l	671	22.9	66.000	
	>5.5 mg/l	1027	41.1	41.000	
LDH	normal	1479	70.9	63	<0.001
	abnormal	607	29.1	78	
Type	IgG	1346	53.1	64	
	IgA	647	31.8	46	
	IgD	76	3.0	28	
	IgE	4	.2	7	0.89
	IgM	6	.2	8	
	Light chain disease	495	19.5	46	
	Nonsecretory	59	2.3	69	
ISS	I	508	18.6	90	<0.001
	II	972	37.5	56	
	III	1112	42.9	42	
cytogenetics	DIPLOIDY	1161	62.6	52.0	0.295
	HYPERPLOIDY	251	13.6	49.0	
	NHO	442	23.8	41.0	
13q deletion	yes	215	13.0	24.0	<0.001
	no	1405	87.0	52.0	
t(11;14)(FISH)	yes	122	17.6	76.0	0.177
	no	570	82.4	50.0	
t(4;14)(FISH)	yes	71	11.0	24.0	0.079
	no	573	89.0	51.0	
t(14;16)(FISH)	yes	28	5.1	NR	0.354
	no	479	94.9	54.0	
17p13deletion (FISH)	yes	122	14.1	40.0	0.374
	no	743	85.9	52.0	
high risk	yes	607	23.1	41.0	<0.001
	no	2292	76.9	64.0	

Methods. Data were collected from 21 centers from 7 countries and regions were collected retrospectively. Clinical characteristics of 2969 symptomatic MM patients at diagnosis were described. Overall survival (OS) and prognostic factors were analyzed for 2273 patients who have survival data. **Results.** Clinical and genetic characteristics were summarized at table 1. Median OS was 54 months (95% CI 48.0-60.0). Patients who were diagnosed before 2000 were shorter survival. Transplantation was performed to 513 patients with better survival (84 vs. 45 months, p<0.001). First line treatment of 2339 evaluable patients was analyzed. Overall response rate was 71% with 30%, VGPR or better. New drugs including bortezomib, thalidomide or lenalidomide were combined for 32.5% of all 2339 patients without difference in response rate or OS according to combination. **Conclusions.** We successfully described clinical characteristics of Asian MM patients and this project will be the base for future studies or clinical trials for Asian MM patients. Updated analyses and comparison with Western data will be presented. AMN, supported by the International Myeloma Foundation (IMF) IMWG initiative.

0838

AN INTERNATIONAL SURVEY INTO THE MANAGEMENT OF MYELOMA KIDNEY

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Introduction: A rapid diagnosis and reduction of serum free light chains (FLC) is essential for improving outcomes in patients with acute kidney injury secondary to multiple myeloma. The development of new generation high cut-off (HCO) haemodialysers with increased pore sizes has improved the ability to remove FLC in these patients. This study assesses how new high cut-off (HCO) dialysers are being used internationally to complement chemotherapy and the outcomes associated with their use. **Methods.** All clinicians that have treated patients with HCO dialysers were invited to take part in an international chart audit, using an electronic web-based data collection system.Results. To date, data from 60 patients, in 22 centres across 11 countries, has been included. All patients were Caucasian with a median age of 65 years (range 42-81). 79.3% of the population received renal biopsies at presentation, with 88.9% having myeloma kidney as their primary diagnosis. Baseline serum biochemistry was: creatinine 642.0umol (range 168-2263); eGFR 8 (1-27); calcium 2.32mmol (0.91-3.83); albumin 34g/L (14-46) and B2M 8.8mg/L (0-55.7). All patients had serum FLC measurements, 57.4% had lambda (median baseline concentration 4165mg/L (300-38000mg/L)) and 42.6% had kappa monoclonal proteins (median baseline concentration 5130mg/L (2250-63200mg/L)). In total 670 dialysis sessions have been recorded, with a median of 13 sessions per patient. The majority of patients (68.6%) were treated with the Theralite® dialyser, 25.7% were treated with the smaller HCO1100 dialyser and only 5.7% with 2xHCO1100 in series. 62.5% of patients received a bortezomib-based chemotherapy regimen, with only 10.4% receiving Thalidomide-based. The median reduction in serum FLCs per treatment was 92.4% (range 4.31-99.9). By day 12 the median reduction in FLCs was 76.0%. Patients treated with Theralite® had a significantly greater FLC reduction by day 12 (median 86.57%) compared to patients treated with HCO1100 (54.45%, Mann Whitney U p=0.019). Independence of dialysis was achieved in 58.3% of patients, at median time of 26 days (range 8-249). More patients treated with Theralite® became independent of dialysis (73.9%, median 24.5days) than those treated with the HCO1100 (50%, median 66.5days). Independence of dialysis was greater in those patients in whom there was a reduction in serum FLCs by day 12 (Mann Whitney U p=0.016).Discussion: Early reductions in serum FLC was associated with an increased likelihood of achieving dialysis independence. To achieve this rapid diagnosis and effective treatment is essential. In patients with AKI secondary to multiple myeloma, the extracorporeal removal of FLC by HCO dialysis complements effective chemotherapy treatment to improve patient outcomes.

0839

IDENTIFICATION OF PROGNOSTIC FACTORS FOR MALIGNANT TRANSFORMATION IN PERSONS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE OBSERVED IN THE CZECH REPUBLIC

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Background. Monoclonal gammopathy of unknown significance (MGUS) is a potentially malignant condition associated with a cumulative probability of progression into one of the malignant forms of monoclonal gammopathy approximately 1% per year (Kyle et al., New Engl J Med 2002). **Aims.** The aims of this study were to verify the validity of already known risk factors separating benign and malignant MGUS using Register of Monoclonal Gammopathy (RMG) of Czech Myeloma Group. **Methods.** Before inclusion to RMG all persons signed informed consent. Total of, 1448 persons with MGUS were enrolled to the RMG in the Czech Republic retrospectively and prospectively, from May 2007 to November 2011. A total of 99,3% (1439/1448) persons were analyzed. The median follow up was 5 years (range 1.0 to 18.0). **Results**. Total of 7,1% (96/1356) persons with MGUS progressed into malignant form; 75% (72/96) into multiple myeloma; 10,4% (10/96) into Waldenström macroglobulinemia; 5,2% (5/96) into malignant lymphoma; 2,1% (2/96) into primary amyloidosis; 7,3% (7/96) into other types of malignancy with the median time to transformation 3 years (range 0-15 years). Variables associated with significantly higher risk of transformation were as follows: serum paraprotein levels >15 g/l (25,8% vs. 4,48%; p< 0.001), bone marrow plasma cells infiltration >5% levels (22,3% vs. 5,4%; p< 0.001), abnormal κ/λ index < 0,26 or > 1,65 (75,6% vs. 42,7%; p< 0.001), serum baseline lactate dehydrogenase levels >3,75 ukat/l, (7,82% vs. 4,62%; p= 0.016) and serum baseline hemoglobin levels <120 g/l (7,82% vs. 4,62%; p= 0.007). In contrast to stratification system published by Rajkumar et al.(Blood 2005), we did not find any correlation between Ig isotypes (non IgG vs. IgG) (32,6% vs. 29,5%; p= 0.523), however high-risk subgroup (presence of all three abnormal risk factors) belonged to a group with a relative risk (Hazard Ratio; HR) almost 18 times higher than normal (17.54; CI (5.78; 53.22); p<0,001) when Cox model was used to identify possible predictors of malignant transformation. High HR (4.18, CI (2.59, 6.74), p <0.001) also had a number of plasma cells greater than 5% levels, serum paraprotein levels >15g /l (HR 3.64, CI (2.39, 5.54) p <0, 001) and pathological values of the κ/λ index HR was 2.9 (2.90 (1.47, 5.75), p = 0.002). Persons in the lowest risk group had 5-year survival without malignant transformation in 96,7% of cases with an annual risk of transformation under 1%, while the highest risk group in 55,6% of cases with and annual risk of transformation about 8-9%. **Conclusions.** Our results confirm the validity of the known risk factors, except type of Ig for prediction of MGUS transformation into malignancy. Currently, we can well define the group with the lowest risk with an annual risk of transformation < 1%. Further optimization of the currently used model of stratification is required mainly for other than low risk groups. At this point, our data form one of the largest set of analyzed subjects with MGUS in the world; however, a certain limitation is a short median follow-up.

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0840

POTENT INHIBITION OF SELECT PROTEASOME SUBUNITS BY CARFILZOMIB IN MULTIPLE MYELOMA AND SOLID TUMOR PATIENTS IS ASSOCIATED WITH PATIENT RESPONSE

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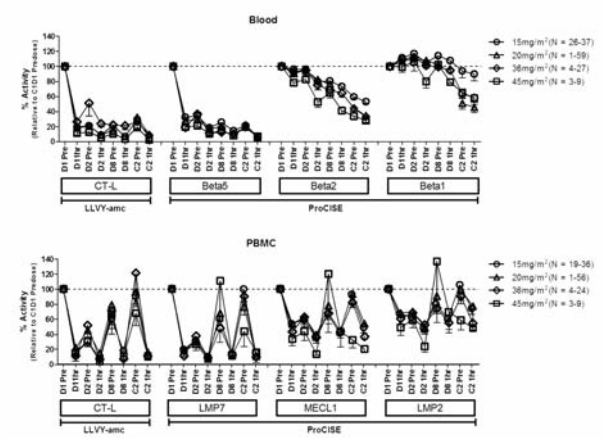
Background. Carfilzomib is a next-generation proteasome inhibitor with demonstrated anti-tumor activity in multiple myeloma (MM). MM tumor cells express both the constitutive proteasome (c20S) and immunoproteasome (i20S), but inhibition of individual active-site subunits has not been determined due to the lack of effective tools. Current assays have been limited to the chymotrypsin-like (CT-L) subunits and do not differentiate between the two proteasome types. In MM cells, both proteasome types are necessary for tumor cell survival. We analyzed proteasome inhibition by carfilzomib in patient samples using a recently validated assay that differentiates between inhibition of the two proteasome types as well as individual active sites. **Aims.** To 1) measure the inhibition of all 6 proteasome active sites in whole blood and peripheral blood mononuclear cells (PBMC) from patients treated with carfilzomib in clinical trials, 2) demonstrate proteasome inhibition in CD138+ tumor cells isolated from carfilzomib-treated patients, and 3) determine if a relationship exists between patient response and proteasome inhibition. **Methods.** ProCISE (Proteasome Constitutive Immuno Subunit ELISA) is an ELISA-based assay that accurately and reproducibly measures proteasome subunit levels and inhibition by clinically applied inhibitors. Whole blood and isolated PBMC were obtained from patients enrolled in one Phase 1b, 1 Phase 1b/2 and 3 Phase 2 trials utilizing carfilzomib by 2- to 10-minute or 30-minute infusion (single agent or in combination with lenalidomide and dexamethasone). Whole blood and isolated PBMC were obtained from 41 solid tumor (ST) and 73 MM patients enrolled in clinical trials. Blood samples were taken on multiple days during the first cycle and on day 1 of the second cycle. Bone marrow aspirates were obtained at screening and after the second dose of carfilzomib and enriched for $CD138^+$ tumor cells. Proteasome inhibition was measured by ProCISE across multiple time points and compared to best overall response rate (ORR).



Results. Carfilzomib administration induced consistent and potent inhibition of CT-L subunits of both the c20S (b5) and i20S (LMP7) and inhibited all subunits of the i20S (LMP7, LMP2 & MECL1). Across all tested doses, CT-L inhibition reached >80% (Figure). In CD138+ tumor cells, we measured CT-L and MECL1 activity and found that inhibition levels corresponded to that in PBMC. Proteasome inhibition was not affected by concomitant medication (high-dose lenalidomide) or renal function. Significantly greater inhibition was observed on cycle 1 day 8 for all i20S subunits 1hr post-dose in patients receiving 56 mg/m^2 carfilzomib compared to patients receiving lower doses (15 or 20 mg/m^2). A

64% ORR was observed in patients receiving 56 mg/m^2 carfilzomib, achieving >80% total i20S inhibition while the lower doses were associated with an 11% ORR and <50% total i20S inhibition. **Summary and Conclusions.** ProCISE represents the only known assay capable of measuring the activity of all 6 proteasome active sites in complex biologic samples. Carfilzomib administration results in potent and prolonged inhibition of multiple proteasome active-sites in patients with MM. By using ProCISE, we have demonstrated that higher doses of carfilzomib are associated with greater levels of immunoproteasome inhibition as well as improved clinical response.

0841

EVALUTION OF BENEFITS AND POTENTIAL ANTIMYELOMA EFFECT OF ZOLEDRONIC ACID IN PATIENTS WITH ASYMPTOMATIC BIOCHEMICAL RELAPSES

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Introduction: Mutltiple myeloma (MM) biochemical relapses after prior responses are usually not treated in the absence of symptoms. Such patients are an ideal group to explore the antitumor benefit of Zoledronic Acid (ZOL) in the absence of any other cytotoxic therapy.**Aims.** To evaluate the potential antitumor effect of ZOL treatment in MM patients under asymptomatic biochemical relapse. Primary end-point was Symptomatic Progression Free Survival (sPFS). Secondary evaluation variables were response rate, skeletal related events and time to next chemotherapy.**Patients and methods**: 192 patients are calculated to be recruited in a randomized, prospective, open label phase IV trial in which a group of patients receive ZOL (4 mg iv./4 wk, 12 doses) and Best Supportive Care (BSC) and the rest only BSC. All patients are monitorized every 4 wk.**Results.** This is an interim analysis corresponding to the first 75 patients included in the trial: 37 treated with ZOL and 38 without ZOL. All patients were in Asymptomatic Biochemical Relapse, with a median age of 70 yr (37-85) and a male female distribution of 39/36. M-component distribution was IgG (70%), IgA (26%) and only light chain (4%). Relapse had presented after 1, 2 or ³3 lines of therapy in 67%, 23% and 10% of cases, respectively. Prior treatment had included transplant (59%), bortezomib (59%) or IMiDs (38%), or a combination of them. One or two skeletal related events (SRE) were detected in 32% of cases. FISH/cytogenetics was abnormal in 52% of cases: t(11;14) 19%, Rb deletion (alone) 17%, del(p53) 8%, t(4;14) 4% and t(14;x) 4%. After randomization, both groups of patients were well balanced in terms of prognostic features, prior response, and time from diagnosis and relapse to the inclusion in the trial. 12 patients have completed the program and four terminated before completion due to patient refusal (n=2) and development of other diseases (n=2). 30 patients are still ongoing and 29 have progressed before 12 mo of treatment, rendering median sPFS of 307 days (10.1 months) with no statistically significant differences between the two arms, although the 12-month projected sPFS was slightly better in patients treated with ZOL vs. patients not treated with ZOL (55% vs. 42%, p>0.1). Interestingly, the patients not treated with ZOL progressed with more advanced bone disease (6 cases of new bone lesions or re-growth of prior lesions, 1 spinal cord compression, and 2 cases of hypercalcemia) vs. patients treated with ZOL (two cases regrowth of bone lesions, p<0.01). By contrast, patients treated with ZOL progressed more frequently with decrease of Hb level below 10 g/dL (11 vs. 7 cases). There were 7 SRE that occurred only in the group of patients treated without ZOL (p=0.006)**Conclusions**: Zoledronic Acid therapy in MM with asymptomatic relapse seems to reduce the risk of progression with symptomatic bone disease and SREs. The possible antitumor effect of Zoledronic Acid alone in biochemical relapses cannot be elucidated yet. This interim analysis supports the continuation of the trial in order to include a highernumber of patients and longer follow-up.

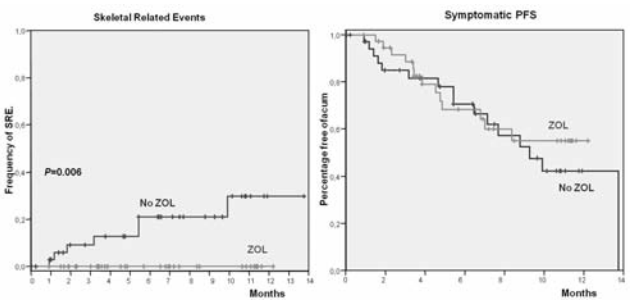

Figure 1. SRE and PFS according to the therapeutic arm.

0842

IS MULTIPLE MYELOMA A CHRONIC DISEASE?

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Background. The introduction of novel agents (Bortezomib, Thalidomide, Lenalidomide) in Multiple Myeloma (MM) has been shown to increase overall survival (OS). In clinical studies the effect of those drugs are always compared to a MM population and not an age and gender matched normal population. A question that has been asked is if MM could be regarded as a chronic disease after the introduction of the novel agents. We define a chronic disease to have a similar survival curve as a matched population without the disease under investigation. **Aims.** Evaluate OS outside clinical studies in non-HDT patients with MM. Comparing the effects of novel agents in 1st line vs. standard chemotherapy. Compare novel agents in 1st line vs. a gender and age at the time for diagnosis matched Swedish normal population to investigate if MM could be regarded as a chronic disease. **Methods.** All diagnosed patients with MM between January 2000 and July 2010 at Karolinska University Hospital Huddinge and Södersjukhuset and between January 2005 and July 2010 at Karolinska University Hospital Solna were included. Standard statistical methods were used. The entire Swedish population was included to select a gender and age at diagnosis matched cohort in 5-years age strata. All individuals matching the criteria were included. Actual observed death rates were used.

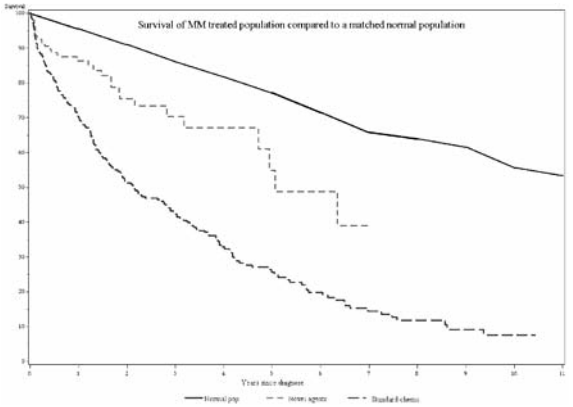

Figure 1. Survival of MM treated population in 1st line with standard cheomtherapy, novel agents and an age at diagnosis and gender matched normal population.

Results. Our MM study population consisted of 674 patients of which 89 patients (13%) were excluded due to non treatment demanding disease or plasmacytoma without MM diagnosis and 214 patients (32%) receiving high

dose treatment, setting the MM study population to n=371. The median age was 75 years and 51% male. The median follow up of the study population was 54.3 month. The most common type of MM was IgG (56%) followed by IgA (25%) and Bence Jones (15%). At diagnosis 59% of the patients had one or more skeleton destruction and 18% had a creatinine higher than 177 µmol/l. Patients treated with novel agents in the first line had a statistically significant lower creatinie 130 µmol/l vs.188 µmol/l compared to patients treated with standard chemotherapy. The use of novel agents in the 1st line predicted a longer OS, median 61.2 month vs. 26.4 month (p<0.001) compared to standard chemotherapy. In the matched normal population the median survival was not reached. When comparing patients treated with novel agents in 1st line to the matched normal population the 1-year survival was 86% vs. 96%, 3-year survival 69% vs. 86% and 5-year survival 52% vs. 77%. **Summary.** The patient cohort treated with novel agents in 1st line has a superior survival compared to the group treated with standard chemotherapy. In comparison to a gender and age at diagnosis matched normal population, the survival is still shorter. However, if novel agents were used in an optimal treatment sequence the survival could potentially be higher but there is still a need for further development in MM treatment before we can call it a chronic disease.

0843

PHASE I/IB STUDY OF AUY922 ALONE OR WITH BORTEZOMIB, IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background. The molecular chaperone HSP90 plays a pivotal role in the folding and maturation of proteins important in myeloma cell survival and hence multiple myeloma (MM) pathogenesis. AUY922 is a non-geldanamycin HSP90 inhibitor with potent preclinical activity against MM cell lines (IC_{50} <12.5 nM) (McMillin *et al.* ASH 2007). **Aims.** This open-label Phase I/Ib study assessed AUY922 as single agent or in combination with bortezomib (BTZ) in patients with relapsed or refractory MM. **Methods.** In the Phase I dose-escalation part, an adaptive Bayesian logistic regression model (BLRM) was employed to guide dose escalation. AUY922 was administered intravenously once-weekly in patients with MM who progressed following 2"'4 prior lines of therapy. In the Phase Ib part, AUY922 was administered in combination with BTZ in patients with MM who progressed following ≤2 prior lines of therapy. The primary objective was to determine the maximum tolerated dose (MTD) of AUY922 alone and in combination with BTZ in patients with previously treated MM. Secondary objectives included preliminary efficacy, safety, and pharmacokinetics (PK). Informed consent was obtained from all patients. **Results.** Phase I: 24 patients (median age 63.5 years; 63% male; 96% ECOG PS 0"'1) received AUY922 at doses of 8"'70 mg/m^2. Most patients were heavily pretreated: 79% had received >2 prior lines of antineoplastic therapy. One dose-limiting toxicity (DLT) was observed (Grade 3 blurred vision at 70 mg/m^2) and no MTD was reached. The recommended Phase II dose was 70 mg/m^2, which was identical to that declared in solid tumor studies (Samuel, *et al.* ASCO 2010). A best response of stable disease (SD) was achieved in 16/24 (67%) patients. There were no partial responses or complete responses.Phase Ib: 5 patients (median age 68 years; 40% male; 100% ECOG PS 0"'1) were treated with AUY922 50 mg/m^2 + BTZ 1.3 mg/m^2. All patients had received 1 line of previous antineoplastic therapy. Three patients experienced DLT (Grade 3 musculoskeletal pain, Grade 3 diarrhea, and Grade 3 non-cardiac chest and musculoskeletal pain [1 patient each]). No further dose escalation was performed and the study was discontinued; MTD was not established.The most frequent Phase I drug-related adverse events (AEs) of any Grade were diarrhea (46%), visual AEs (37.5%; most frequently night blindness [17%], photopsia [17%] and visual impairment [17%]), and nausea (17%). Grade 3/4 AEs were rare (all <10%). The safety profile was similar to that observed in solid tumor studies. PK profiles were also similar to those reported in previous studies, and drug disposition of AUY922 was unaffected by combination with BTZ. **Conclusions**. AUY922 administered as single agent had an acceptable safety profile. Limited activity was observed: SD was achieved by 67% of heavily pretreated patients with relapsed/refractory MM. Combination with BTZ was not tolerated at the standard recommended dose.

0844

CARFILZOMIB DOSE AND SCHEDULE NEED NOT BE ADJUSTED FOR BASELINE RENAL DYSFUNCTION, INCLUDING PATIENTS ON HEMODIALYSIS

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Background. Patients with multiple myeloma (MM) are vulnerable to renal injury and impairment both from their disease and from treatments for the disease. Carfilzomib is a selective proteasome inhibitor with proven efficacy in MM. Safety data have been compiled from over 700 patients with relapsed and/or refractory MM who have received single-agent carfilzomib.**Aims.** Herein we present an analysis of the incidence and severity of carfilzomib treatment-emergent renal adverse events (AEs) from 526 patients in four phase 2 trials, including a study focused on individuals with varying degrees of renal function, which includes patients on hemodialysis. **Methods.** Patients from the PX-171-003-A0, PX-171-003-A1, PX-171-004, and PX-171-005 trials were included in this analysis. In all studies, carfilzomib was dosed on Days 1, 2, 8, 9, 15, and 16 of a 28-day cycle (C). Doses were 20 mg/m^2 in C1 for all studies escalating to 27 mg/m^2 in C2 per individual protocol, except 005 (15 mg/m^2 in C1, 20 mg/m^2 in C2, and 27 mg/m^2 in C3). Renal AEs"'the incidence, frequency, and resolution of episodes of worsening renal function, defined minimally as a doubling of serum creatinine from baseline"'and shifts in other laboratory parameters were tabulated and summarized based on NCI CTCAE v.3 criteria. **Results**. The majority of patients (71%) had renal dysfunction (CrCl <50 mL/min) at baseline. Overall, 87% experienced no significant worsening of renal function during the course of treatment. Transient worsening was reported in 31 patients (6%) with a median duration of 1.4 weeks and a median of 1.0 episode per patient; non-transient worsening was reported in 37 patients (7%) with 8 (2%) of those permanently discontinuing treatment due to renal dysfunction. 38 patients (7%) experienced Grade 3/4 acute renal failure AEs, of which 31 were Grade 3; 50% of patients with acute renal failure AEs required no change in carfilzomib therapy. The percentage of patients in 003-A0, 003-A1, and 004 whose creatinine levels shifted to Grade 3 or 4 from any lower grade was <5%. Results from 005 showed no major pharmacokinetic differences in patients with a wide range of renal function. **Conclusions**. Treatment-emergent renal AEs resulting in carfilzomib discontinuation were uncommon. Based on the findings from this cross-trial analysis, carfilzomib dose and schedule need not be adjusted in patients with baseline renal dysfunction, including patients on hemodialysis, allowing these patients to receive the benefits of standard carfilzomib treatment.

0845

UPFRONT USE OF NOVEL AGENTS IS BETTER ABLE TO OVERCOME THE ADVERSE IMPACT OF HIGH-RISK MYELOMA: A JOINT ANALYSIS OF THE SINGAPORE MM STUDY GROUP AND THE KOREAN MM WORKING PARTY

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Introduction. Multiple myeloma (MM) is clinically heterogeneous and risk stratification is vital for prognostication and informing treatment decisions. We evaluate the survivaldata of MM patients managed at tertiary referral centers in Singapore (SG) and South Korea (SK) in the current era, where various novel agents were introduced at the same time, but the sequencing of their use in treatment algorithms of MM differs significantly due to differing healthcare reimbursement systems. We sought to determine the impact of an upfront risk-adapted vs a sequential approach to bortezomib (Btz) on the overall survival (OS) of patients. We aim to study the optimal sequencing of novel agents in treatment of MM. **Methods.** From the comprehensive MM registries of the SG and SK MM study groups, we study the survival data of 432 unselected and

previously untreated MM patients diagnosed from 2006 to 2009. Thalidomide (Th), bortezomib (Btz) and lenalidomide became available for treatment of relapsed MM in both countries from 2001, 2005 and 2009 respectively. From 2006 however, pts with high-risk MM (ISS III associated with renal impairment, del (13q) or hypodiploidy on metaphase cytogenetics, or presence of del (17p), t(4;14) or t(14;16) on interphase FISH) in SG could receive frontline Btz-based induction regimens, while Btz could only be approved for second or subsequent line usage in SK. All patients < 65 years were eligible for HDT/ASCT. Baseline pt, disease and treatment characteristics, and survival data were evaluated overall, and with respect to country of treatment. Disease response was assessed by the IMWG criteria after induction treatment and after HDT/ASCT for transplant ineligible and eligible pts respectively. We applied multivariate Cox's regression modeling to determine what baseline parameters, along with the eventual induction response, significantly affected the OS in the respective countries. **Results.** Median age of all patients was 61 years. Although baseline demographic features were comparable between the 2 countries, there were more pts presenting with ISS III disease in SK. Proportion of pts receiving novel agents (Th or Btz) in the frontline were 90% vs 26% in SG and SK respectively. At a median follow-up of 19 months, 47% vs 60% of pts had received Btz in SG and SK respectively. More pts in SG received Btz in the frontline while all pts in SK received Btz at relapse. Proportions of pts receiving HDT/ASCT were comparable. Significantly more pts had no response to induction treatment in SK. The median OS of pts in SG and SK were not reached and 4.83 years respectively (p=0.37). On multivariate analysis stratified by country of treatment, the attainment of ≥VGPR emerged as only significant prognostic factor in SG (p=0.006) while the presence of high-risk ISS and adverse cytogenetics still retains their prognostic significance in SK (p=0.003). **Conclusions**. Our study suggests that in the era of novel therapy, novel agents used upfront rather than sequentially may be better able to overcome the adverse impact of high-risk ISS and adverse cytogenetics. These findings have implications in healthcare resource planning.

0846

VELCADE CYTOXAN DEXAMETHASOE (VCD) OR VELCADE THALIDOMIDE DEXAMETHASONE (VTD) INDUCTION REGIMENS FOR NEWLY DIAGNOSED MULTIPLE MYELOMA - WHICH REGIMEN SHOULD WE PREFER?

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Background. Three drug induction regimens have become the standard of care in patients with multiple myeloma (MM). Two of the most frequently used protocols are Velcade-Cytoxan-Dexamethasone (VCD) and Velcade-Thalidomide-Dexamethasone (VTD). These regimens differ in their response rate, toxicity profile and cost. However, prospective "head to head"comparisons between the two are lacking. **Aims.** In this study we attempted to identify the differences in response and toxicity between the VCD and VTD regimens by reviewing the relevant literature and conducting meta-analysis of the eligible studies. **Methods.** We thoroughly searched MEDLINE, Cochrane library, and the web sites of the American Society of Hematology, the American Society of Clinical Oncology and the European Hematology Association for clinical trials in which VTD or VCD were used as first line induction regimens for newly diagnosed MM patients. A meta-analysis using the Chi square for testing homogeny and odds-ration (OR) with 95% confidence interval to estimate adverse events, overall response rate (ORR), complete remission (CR) rate and near complete remission (nCR) rate, was performed. **Results.** Twelve clinical trials were proved eligible. Overall 807 patients were treated with either VCD (n=170) or VTD (n=637) as induction therapy. After a median of 4 induction cycles the ORR didn't differ between the groups: 85.3% in the VTD and 86.5 % in the VCD group (OR =0.90, CI= 0.52-1.56). VCD protocol was found to be slightly superior in terms of CR/nCR rate: 45% vs. 34% in the VTD arm (p= 0.05). Grade 3-4 adverse events of any type were documented in 68% of patients treated with VCD, compared with only 51% in the VTD group (p=0.01). Interestingly, there was no statistically significant difference in the incidence of grade 3-4 neuropathy (5.4% vs. 5.9%) between the two groups. **Conclusions**. Both VCD and VTD induction regimens in patients with MM are efficacious in term of ORR. VCD regimen may be superior in its ability to achieve CR/nCR; however, grade 3-4 adverse events are more frequently observed with this regimen. Surprisingly, the incidence of grade 3-4 neuropathy was comparable between the two groups. Due to the limited number of trials and their heterogeneity, randomized controlled trials are needed to confirm these results.

0847

BORTEZOMIB-BENDAMUSTINE-DEXAMETHASONE (BBD) IN PATIENTS WITH RELAPSED/REFRACTORY MYELOMA AND DIFFERENT RISK PROFILES NAMELY, CYTOGENETICS, PREVIOUS EXPOSURE TO BORTEZOMIB OR TO LENALIDOMIDE

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Background. To evaluate the efficacy of Bortezomib-Bendamustine-Dexamethasone in relapsed/refractory myeloma in relation to FISH defined cytogenetics, and pre-exposure to Lenalidomide- and Bortezomib-based treatments**.** Outcome of treatment in patients of relapsed/refractory myeloma depends on the efficacy of the rescue regimen, on patient and tumour characteristics and on previous treatments. Bendamustine, an alkylating drug with purine like activities may exert synergistic activity in combination with bortezomib and may render patients previously exposed to bortezomib sensitive to anew treatment with the proteasome inhibitor. **Methods.** 71 patients with relapsed/refractory MM have been enrolled. Median age: 65 years, range 40-86 years, male/female: 32/39, ISS stage*I//III: 22, 29, and 20 patients, respectively. ECOG status* II: 37, 31, and 3 respectively. Previous treatment lines: 1-2: 44, 3-4: 22, and ≥ 5: 5, respectively. Full data documentation for response (>1 cycle) is available for 65 patients. Treatment regimen: Bendamustine 70 mg/m^2 day 1+4, Bortezomib 1.3 mg/m^2 days 1, 4, 8 and 11, Dexamethasone 20 mg on days 1, 4, 8 and 11, repeated every 4 weeks. Treatment was scheduled for 8 cycles, but should be discontinued in case of no response after 4 cycles at latest. Survival curves were calculated using SPSS software (version 17); comparisons were made using the log rank test. All survival data are provided for the intent-to-treat population.

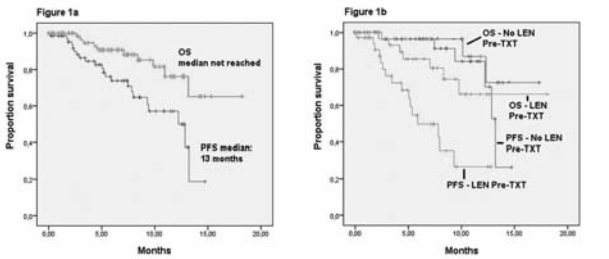

Figure 1a: PFS and OS in all patients; Figure 1b: PFS and OS in Lenalidomide naive and pre-treated patients; (p<0.0001; p=0.074, respectively)

Results. At the time of analysis the median follow up is 7,1 months. 6 of the 71 patients enrolled had less than 1 cycle, while in the entire group the median number of cycles is 4. Overall response rate (CR-PR) was 58.5% and 75.4%, respectively when MR was included (CR-MR) in the patients who completed ≥ 1 cycle. Eleven patients (16.9%) had CR/nCR, 10 (15.4%) VGPR, 17 (26.2%) PR, and 11 (16.9%) MR, respectively. Overall response rate (CR-PR) in patients previously exposed to bortezomib was 56% (CR-MR: 73%) and was 47% (CR-MR: 68%) in those pre-treated with lenalidomide-based regimens.Median progression-free survival (PFS) was 13 months, while for overall survival (OS) the median has not been reached as yet (figure 1a). Interestingly, no difference in PFS (p=0.733) and in OS (p=0.377) was found between patients with and without FISH defined adverse cytogenetics (t(4; 14), del17p, ampl 1q21). In patients pre-exposed to bortezomib, no difference in PFS (p=0.219) and in OS (p=0.378) was noted in comparison to bortezomib-naïve patients. In contrast, pre-treatment with lenalidomide was found to be significantly associated with shortened PFS (p=0.0001), while for OS (figure 1B) no difference was noted (p=0.074).Toxicities: Grade 3/4 anaemia was found in 13%, leucopenia in 14%, thrombopenia in 36%, infections in 15% (plus 3% G5) and PNP in 5% of patients. **Summary.** In conclusion, the BBD regimen showed significant activity in relapsed/refractory myeloma. PFS and OS were similar in patients with and without adverse cytogenetics as well as in those pre-exposed to bortezomib or bortezomib naïve. Lenalidomide pre-treatment, however was associated with significantly shorter PFS. The regimen was well tolerated with mainly haematological side effects and only 5% grade 3/4 PNP.

0848

COMBINED BENDAMUSTINE, PREDNISOLONE AND LENALIDOMIDE (RBP) IN PATIENTS WITH REFRACTORY OR RELAPSED MULTIPLE MYELOMA. FINAL RESULTS OF A PHASE I CLINICAL TRIAL - OSHO - #077

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Background. While the role of lenalidomide monotherapy in the treatment of relapsed/refractory patients with multiple myeloma (MM) is established, combination therapies with lenalinomide are still under investigation. Bendamustine is a bi-functional alkylating agent with a purine-like benzimidazole ring effective in combination with steroids, thalidomide and bortezomib for the treatment of patients with MM. In the current trial, combination therapy of bendamustine, lenalinomide and prednisolone (RBP) was tested for feasibility and safety in patients with relapsed or refractory MM. **Methods.** In this phase I part of the trial the dosing of lenalidomide in combination with bendamustine and prednisolone was examined. The first cohort of patients received a starting dose of 10 mg/d d1-21 lenalidomide, 60mg/m²/d d1-2 bendamustine and 100mg/d d1-4 prednisolone. Escalation steps in the next cohorts included 15, 20 and 25mg of lenalidomide followed by an escalation step of 75 mg/m² bendamustine. 3 (to -6) patients were enrolled at each dose level and the first two cycles were evaluated for maximum tolerable dose (MTD). Patients received RBP in 4-week cycles for a maximum of 8 cycles in order to evaluate efficacy. Patients with stable or responding disease following 8 cycles of RBP received single-agent oral lenalidomide 10 mg once daily on days 1-21 of each 28-day cycle as maintenance. **Results**. 21 patients (3 at the first three dose levels and 6 at the last two dose levels) have been enrolled in this phase I study and all patients have completed at least 2 cycles. Response was assessed using modified EBMT criteria to include near complete remission (nCR) and very good partial remission (VGPR). 16 patients (76%) responded after at least two cycles of RBP with 1 sCR, 1 nCR, 4 VGPR and 10 PR. Three patients experienced a MR and two a SD/PD.Two of the 21 patients developed dose-limiting hematoxicity as defined by an ANC < 1,0 x 10^9/l with fever for > 3 days or an ANC < 0,5 x 10^9/l for > 7 days or platelet count < 25 x 10^9/l for > 3 days: One patient in the dose level with 60 mg/m² bendamustine and 25 mg lenalidomide and one patient in the last dose level with 75 mg/m² bendamustine and 25 mg lenalidomide. The study finished with 75 mg/m^2 for bendamustine and 25 mg for lenalidomide without reaching MTD. **Conclusions.** RBP with a dose of 25 mg lenalidomide d 1-21 and 75 mg/m² bendamustine d 1-2 is well tolerated in patients with relapsed or refractory MM. The study is ongoing and 29 additional patients will be enrolled in the phase II study at the last dose level to better evaluate toxicity and clinical activity.

0849

COMPARISON OF THE CKD-EPI, MDRD AND A FORMULA BASED ON CYSTATIN-C AND SERUM CREATININE FOR THE ESTIMATION OF GFR IN PATIENTS WITH MULTIPLE MYELOMA; IS IT TIME TO CHANGE FROM MDRD TO CKD-EPI EQUATION?

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Background. Renal impairment (RI) is a frequent complication of multiple myeloma (MM). The IMWG has recommended the use of the MDRD formula for the estimation of glomerular filtration rate (GFR) in MM patients with stabilized serum creatinine (sCr). Because MDRD equation has limitations, other prediction equations based on serum cystatin-C (cys-C), a very sensitive GFR surrogate marker, have been suggested for use in patients with chronic kidney disease (CKD). Furthermore, the CKD Epidemiology Collaboration (CKD-EPI) has proposed a new formula for the estimation of GFR, which is based on age, race and sCr, and is more accurate than the MDRD in the estimation of GFR in CKD and in kidney transplant patients. **Aims**. The aim of this study was to evaluate the renal function of newly diagnosed patients with symptomatic MM using the MDRD, the CKD-EPI equations and an equation based on Cys-C/age/sCr (Stevens et al, Am J Kidney Dis 2008;51:395-406) and explore their prognostic value on survival. **Methods.** We studied 204 newly-diagnosed, previously untreated, symptomatic MM patients. Their median age was 69 years (range: 36-94 years); 16% of patients had sCr ≥2 mg/dl, while 59% had elevated Cys-C. **Results**. The median values for eGFR calculated by the MDRD, the Cys-C/age/sCr and the CKD-EPI equations were 63.95 ml/min/1.73m^2, 68.08 ml/min/1.73m^2 and 56.5 ml/min/1.73 m^2, respectively (p<0.01 for all comparisons between equations). Patients were divided in the 5 CKD stages of the KDIGO classification. For each studied equation, the number of patients with RI stage 3-5 (i.e. eGFR <60 ml/min/1.73^2) was 42%, 41% and 52% for MDRD, Cys-C/age/sCr and CKD-EPI, respectively (p<0.001; see also the table).

Table. Evaluation of Renal Function Stage by Different Equations

CKD stage	MDRD equation	Equation based on Cys-C/age/sCr	CKD-EPI equation	p-value
1	47 (23%)	51 (25%)	30 (15%)	
2	70 (34%)	68 (33%)	66 (32%)	Friedman-test
3	54 (26%)	54 (26%)	68 (33%)	p<0.001
4	22 (11%)	23 (11%)	23 (11)	
5	11 (5%)	8 (4%)	17 (8%)	

Concordance for CKD stage allocation for the three equations was 68% for MDRD *vs.* CKD-EPI and Cys-C/age/creatinine and 61% for CKD-EPI *vs.* Cys-C/age/sCr. A significant correlation was found between ISS stage and each of the three equations (p<0.001). The median survival for all patients was 49 months. By using the eGFR of 60 ml/min/1.73 m^2 as a cut-off, patients with eGFR <60 ml/min/1.73 m^2, assessed by each of the 3 studied equations, had a significantly shorter median overall survival: 24 months *vs.* 98 months (χ^2=9.8, p=0.002) for MDRD equation, 27 months vs. 98 months (χ^2=12.8, p<0.001) for Cys-C/age/sCr equation and 38 months *vs.* not reached (χ^2=13.3, p<0.001) for CKD-EPI equation. When we adjusted for ISS stage, the allocation to RI of stage 3-5, using the CKD-EPI equation was significantly associated with survival (p=0.041); this was not observed for the allocation to stage 3-5 RI using the other formulas (p=0.357 for MDRD equation and p=0.235 for Cys-C/age/sCr equation). **Conclusions.** Our data suggest that CKD-EPI equation for the estimation of GFR detects more MM patients with stage 3-5 RI than MDRD or Cys-C/age/sCr equations. Furthermore, CKD-EPI was the only equation that could predict for overall survival adjusted for ISS stage. The confirmation of these data may lead to the broader use of CKD-EPI formula for the evaluation of RI in patients with MM, as it has been suggested for patients with several renal disorders.

Myeloma - Clinical 3

0850

IMPACT OF NEUROPATHY ON STEM CELL MOBILIZATION IN PATIENTS WITH PLASMA CELL DISORDERS

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Background. Number of autologous stem cell transplants (ASCT) being done for treatment of Multiple Myeloma (MM) and Amyloidosis has increased in the past decade. Nearly 10% of patients fail to mobilize adequate number of cells to proceed to SCT. Multiple factors contribute to poor mobilization including previous therapy, age, degree of disease control and type of mobilization regimen. Sympathetic nervous system plays critical role in the control of stem cell niche as has been described in mouse models. Patients with MM and amyloidosis may have neurological involvement either due to the plasma cell disorder or as a side effect of the treatment for their disease. We hypothesized that patients who have peripheral neuropathy at time of stem cell collection may have a poorer stem cell collection as compared to those without. **Methods.** Study included a cohort of consecutive 906 patients with MM or/and Amyloidosis seen at Mayo Clinic Rochester from December 1999 to October of 2008 who underwent stem cell collection. All patients were mobilized using G-CSF. Data was abstracted using chart review. We analyzed the difference in number of stem cells collected (CD34cells/kg) between patients with neuropathy at the time of collection versus those without. **Results.** Median age was 58 years (range 20-77). Of a cohort of 906 patients; 567 (62%) had MM, 340 (38%) had amyloidosis and 277 (31%) had a diagnosis of neuropathy at the time of stem cell collection. This was either a previous diagnosis, related to their disease or a side effect of treatment. There was no difference in rates of neuropathy based on time from diagnosis. Among patients with amyloidosis there was no difference in stem cell collection based on presence of neuropathy (Days 1-3 collection 5.59×10^6 vs 5.81×10^6; p=0.31). However, among patients with MM with neuropathy, stem cell collection on Day 1 was significantly lower as compared to those without (2.10×10^6 vs 2.56×10^6; p=0.027). Combined total stem cell collection (Days 1-3) was also significantly lower in patients with neuropathy (3.0×10^6 vs 3.7×10^6 p=0.016). Previous studies have shown that lenalidomide significantly lowers stem cell collection. Our data confirmed the same with significantly lower stem cell collection in the cohort treated with lenalidomide (2.24×10^6 vs 3.79×10^6, p<0.0001 for Days 1-3). After excluding patients who received lenalidomide, the impact of neuropathy on stem cell collection in MM patients was more pronounced: Day 1 collection was 2.12×10^6 vs 2.72×10^6, (p=0.009); and collection for Days 1-3 was 5.74×10^6 vs 7.23×10^6 (p=0.003) for those with and without neuropathy. **Conclusions.** Peripheral neuropathy in patients with plasma cell disorders is associated with poor stem cell collection. The impact of peripheral neuropathy may reflect disruption of autonomic nervous system, which may interfere with stem cell mobilization form the marrow niche. Studying role of adrenergic agonists in patients with neuropathy may be worth investigating to see if that enhances the stem cell collection.

0851

BONE MARROW BIOPSY SHOULD BE CONSIDERED FOR THE INITIAL EVALUATION OF INDIVIDUALS WITH ASYMPTOMATIC MONOCLONAL GAMMOPATHY AND IMMUNOPARESIS OR MONOCLONAL COMPONENT =1GR/DL

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Background. The incidental finding of a monoclonal gammopathy is increasingly common. It has been proposed that a bone marrow (BM) aspirate and biopsy is indicated when the monoclonal protein (M-protein) is ≥1.5 g/dL, when abnormalities are noted in the FBC and in serum creatinine, serum calcium, or radiographic bone survey, in individuals with non-IgG monoclonal gammopathy and in those with an abnormal serum free light chain ratio. **Aims.** To identify factors that could aid in the evaluation of individuals presenting with asymptomatic monoclonal gammopathy and in whom invasive diagnostic testing with a BMbiopsy is considered. **Methods.** We analyzed the database of patients who were referred to our department (University of Athens, Greece) for evaluation of asymptomatic monoclonal gammopathy. A BM trephine biopsy, a serum and urine protein electrophoresis with immunofixation and quantitative immunoglobulins are routinely performed in all patients with monoclonal gammopathy. Patients with a monoclonal M-protein ≥3 g/dl (30 g/L), i.e. those diagnosed with asymptomatic myeloma or Waldenstrom's macorglobulinemia based on the standard criteria, were not included in the analysis. BM plasma cells or lymphoplasmacytic cells clonality was assessed by immunohistochemistry. Patients who eventually were diagnosed with plasma cell related conditions (i.e. amyloidosis, peripheral neuropathy, etc.) were also excluded from the analysis. **Results**. Our analysis included 162 patients (females 53%, median age 64 years, range 33-89 years); 53% had a monoclonal IgG, 15.5% IgA, and 24% IgM, while 4% had a biclonal paraprotein; 63% of patients had a kappa light chain. The median serum M-protein was 0.95 g/dl (range 0.1-2.99 g/dl) and was higher in those with IgG or IgA *vs.* those with IgM (p=0.009). Fifty-two percent had an M-protein <1 g/dl and 21% had ≥2 g/dl. Immunoparesis of at least one of the uninvolved immunoglobulins was present in 38% and of both of the uninvolved immunoglobulins in 6% of patients. Median infiltration by monoclonal cells in BM biopsy was 7% for those with an IgM-gammopathy and 15% for those with monoclonal IgG or IgA (p=0.047). There was a significant correlation of the size of M-protein and of the infiltration of the BM (R=0.592, p<0.001). Among those with M-protein<0.5 g/dl, 11% had≥10% clonal cells in their BM biopsies while the respective rates were 88% for those with M-protein ≥1 g/dl and 97% for M-protein ≥2 g/dl. Immunoparesis of at least one of the uninvolved immunoglobulins was associated with ≥10% BM clonal cells in 90% of patients. Light chain isotype, age and gender were not predictive of BM infiltration. In regression analysis, immunoparesis of at least one of the uninvolved immunoglobulins (OR:6.45, 95%CI:2.32-18, p<0.001), an IgG or IgA monoclonal protein (OR:2.67, 95%CI:1.1-6.4, p=0.028) and an M-protein ≥1 g/dl (OR:5.4, 95%CI:2.23-13) were independently associated with ≥10% clonal infiltration in BM biopsy. **Conclusions**. Our data, from a large number of individuals with asymptomatic monoclonal gammopathy who underwent a BM biopsy, indicate that BM biopsy can reveal asymptomatic myeloma or WM who may escape identification with standard criteria and should be included in the standard initial workup of individuals with asymptomatic monoclonal gammopathy.

0852

NORMALIZATION OF SERUM FREE LIGHT CHAIN KAPPA/LAMBDA RATIO IS STRONGLY ASSOCIATED WITH FAVORABLE OUTCOME IN PATIENTS WITH MULTIPLE MYELOMA

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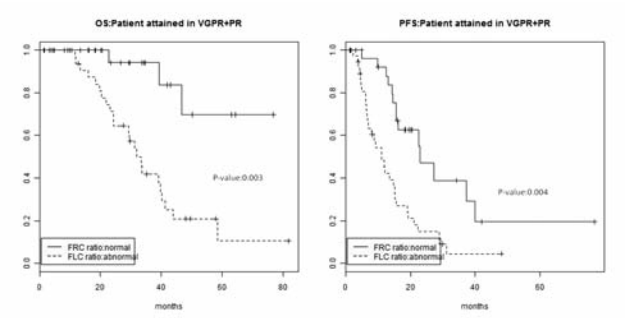
Background. Although serum free light chain kappa/lambda ratio (sFLCk/l) has been incorporated into the IMWG response criteria of myeloma, its attainment in prognosis remains controversial. **Aims.** We investigated the prognostic impacts of normalization of sFLCk/l on progression-free survival (PFS) and overall survival (OS) among patients with MM treated with novel agents. **Patients and Methods.** A total of 104 consecutive patients (58 male and 46 female, median age 71) treated at Kameda General Hospital, Kamogawa-shi, Japan, between April 2005 and January 2012 were analyzed. All patients received chemotherapy containing at least one novel agent, including thalidomide, bortezomib, and lenalidomide. Treatment response was analyzed by bone marrow aspiration and/or biopsy, serum immunofixation, sFLC test, and assessed by IMWG criteria. PFS and OS were calculated from time of diagnosis until date of relapse or death from any cause/date on which the patient was last known to be alive, respectively. **Results.** At a median follow-up of 24 months, 3- and 5-year PFS and OS rates of all patients were 35%, 28% and 61%, 45%, respectively. CR was obtained in 22% of patients (23/104), very good partial response (VGPR) in 31% (32/105), partial response (PR) in 34% (33/104), and stable disease (SD) or less in 13% (14/104). Kaplan-Meier estimated 3-year PFS and OS were 78% and 100 % in patients with CR, which were significantly superior to those in patients with VGPR (PFS 23%, OS 65%), PR (PFS 0%, OS 44%), and SD or less (PFS 0%, OS 0%). Normalization of sFLCk/l was attained in 45 patients (43%) who showed significantly longer survival than 59 patients who did not (3-year OS, 94% vs. 48%; $P < 0.001$). Normal sFLCk/l was attained among patients with 80% of CR, 51% of VGPR, and 6.6% of PR, and 0% of SD or less. Although CR patients who attained normal sFLCk/l did not show significant survival advantage in PFS and OS, patients with VGPR or less with normal sFLCk/l showed significantly longer PFS and OS than those without normal sFLCk/l ($P < 0.001$). Univariate analysis showed that patients age > 70 and abnormal LDH showed negative prognostic impacts on attaining normal sFLCk/l. On multivariate analysis, none of these factors remained significant. Proportional hazard Cox models showed achievement of immunofixation-negative CR and normalization of sFLCk/l ratio were emerged as positive prognostic predictors for longer PFS and OS in patients with MM. **Conclusions.** This study confirmed the importance of immunofixation-negative CR for longer PFS and OS. We also demonstrated the significance of obtaining normal sFLCk/l ratio not only in patients with CR but also in those with

VGPR or PR. The data presented here indicated that analysis of sFLCk/l could identify the favorable group of patients independent of immunofixation results, and support the inclusion of sFLCk/l test as part of the response criteria for MM.



0853

EFFICACY AND SAFETY OF LENALIDOMIDE 25 MG IN ELDERLY PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA AND NORMAL RENAL FUNCTION

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Background. Lower doses of some multiple myeloma (MM) treatments, including lenalidomide, have been suggested when treating elderly patients. However, lenalidomide 25mg plus dexamethasone is shown to be effective in elderly patients with MM when closely monitoring renal function (Touzeau, Leukemia & Lymphoma 2012). Evidence suggests that any dose reduction after ≥12 months of lenalidomide 25mg in patients of all ages significantly reduces the risk of myeloma disease progression (Dimopoulos, Leukemia 2011). **Aims.** To investigate the efficacy and safety of a 25mg starting dose of lenalidomide in elderly relapsed or refractory MM patients with normal renal function. **Methods.** Pooled analysis of patients treated with lenalidomide (25mg on days 1-21 of a 28-day cycle) plus dexamethasone (40mg on days 1-4, 9-12, and 17-20 for the first 4 cycles; thereafter on days 1-4 only) was conducted from two pivotal phase III trials (MM-009 and MM-010). Patients with a creatinine clearance (CrCl) ≥60mL/min (based on the Cockroft-Gault equation) were categorized into those aged <65, 65-75, and >75 years. Efficacy outcomes were overall response rate (defined as partial response or better), time-to-progression (TTP), progression-free survival (PFS), and overall survival (OS). TTP, PFS, and OS were evaluated using Kaplan-Meier survival curves and a log rank test. Age was evaluated as a predictor of PFS in both univariate and multivariate Cox proportional hazards models adjusted for other baseline covariates. Safety outcomes included grade 3-4 adverse events (AEs) and discontinuations due to AEs. Dose intensity (calculated as total dose received/expected total dose) and dose reductions were assessed. **Results.** A total of 239 patients were included: 158 were aged <65 years, 70 were aged 65-75 years, and 11 were aged >75 years. Baseline characteristics were balanced across the groups, with the exception that older patients were more likely to have an ECOG performance score ≥1 (43% aged <65 years, 69% aged 65-75, and 82% aged >75), and younger patients were more likely to have received prior stem cell transplantation (67%, 44%, and 9%, respectively). Despite this, efficacy outcomes were similar across the 3 age groups (p=0.40, 0.38, and 0.81 for TTP, PFS, and OS, respectively). Age as a continuous variable was not a significant predictor of PFS in Cox regression analyses (p=0.53 for the univariate model and p=0.43 for the multivariate model). The incidence of grade 3-4 neutropenia, infection, fatigue, anemia, thrombocytopenia, and neuropathy was similar across all age groups. The rates of dose reduction and discontinuation due to AEs were also comparable across groups (Table). Dose intensity across the age subgroups was: 64%, 59%, and 59% in patients aged <65, 65-75, and >75 years, respectively (Table 1). **Conclusions.** Despite the small number of patients aged >75 years, these results suggest that a lenalidomide starting dose of 25mg (days 1-21 of a 28-day cycle) is effective and adequately tolerated in elderly MM patients with normal renal function, regardless of age. However, careful monitoring and dose adjustments are warranted at the physician's discretion, especially for patients >75 years.

Table 1. Outcomes in elderly patients with relapsed or refractory multiple myeloma and normal renal function (creatinine clearance ≥60mL/min).

	<65yr (n=158)	65-75yr (n=70)	>75yr (n=11)
ORR (≥PR), n (%)	98 (62)	46 (66)	9 (82)
Median TTP, mo (95% CI)	12.1 (9–18)	13.8 (6–19)	17.9 (6–NR)
Median PFS, mo (95% CI)	11.1 (8–15)	12.3 (6–19)	17.9 (6–NR)
Median OS, mo (95% CI)	46.3 (39–54)	39.8 (30–NR)	NR (19–NR)
Gr. 3-4 AEs, n (n/100 pt-mo)			
Neutropenia	20 (0.7)	26 (2.5)	3 (1.7)
Anemia	4 (0.1)	6 (0.6)	2 (1.1)
Thrombocytopenia	6 (0.2)	9 (0.9)	1 (0.6)
Febrile neutropenia	2 (0.1)	4 (0.4)	2 (1.1)
VTE	6 (0.2)	13 (1.2)	1 (0.6)
Neuropathy	1 (0.0)	3 (0.3)	1 (0.6)
Len dose reductions, n (%)	52 (33)	30 (43)	7 (64)
Median time to first dose reduction, mo (median, 25%, 75%)	6.5 (3, 14)	2.9 (2, 9)	9.5 (2, 21)
Discontinuations due to AE, n (%)	20 (13)	11 (16)	2 (18)
Within first 3 mo	10 (6)	4 (6)	1 (9)
Median dose intensity, % (median, 25%, 75%)	64 (49, 74)	59 (45, 68)	59 (45, 70)

0854

A STATISTICAL COMPARISON OF THE EFFICACY AND SAFETY OF LOW-DOSE LENALIDOMIDE + LOW-DOSE DEXAMETHASONE WITH FULL-DOSE LENALIDOMIDE + DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background. Two pivotal phase 3 trials, MM-009 and MM-010 established lenalidomide + dexamethasone (RD) as a standard of care for RRMM patients. However, elderly patients or those at high risk of myelosuppression may be susceptible to toxicities necessitating dose reductions/disruptions and treatment cessation. Interim results from the phase 2 RevLite study suggested that low-dose lenalidomide and low-dose dexamethasone (rd) may achieve similar efficacy with improved tolerability vs RD in patients at high risk of myelosuppression. An initial retrospective comparison with a subset of MM-009/010 patients who met RevLite entry criteria was performed. **Aims**. Compare results from an updated interim analysis of RevLite with those from a subset of MM-009/010 patients who met RevLite enrollment criteria. **Methods.** In RevLite (N = 150), patients received 15 mg lenalidomide for 21 days of a 28-day cycle plus 20 mg dexamethasone on days 1-4, 9-12, and 17-20 for cycles 1-4 and days 1-4 in subsequent cycles until disease progression or unacceptable adverse events (AEs). In the active arm of MM-009/010, patients received a similar schedule, except lenalidomide was given at 25 mg and dexamethasone at 40 mg. A total of 255 RD-treated patients from MM-009/010 were included in this analysis that met ≥ 1 of the following RevLite eligibility criteria: age ≥ 60 years, creatinine clearance (CrCl) ≤ 60 mL/min, platelets ≤ 75 × 10^9/L. Progression-free survival (PFS), time to progression (TTP), and OS were evaluated using Kaplan-Meier methods and the log-rank test. OS was adjusted for baseline covariates using

Cox regression analyses. **Results**. Patient characteristics were similar other than prior thalidomide (rd 65% vs RD 36%). After median follow-up of 18.7 (rd) and 30.0 (RD) months, overall response rates (rd 69% vs RD 60%), median PFS (rd 10.4 vs RD 11 months), and TTP (rd 12.5 vs RD 13.1 months) were similar. Median OS was 30.6 months for rd and 34.8 months for RD, *P* = .02 (log-rank, Kaplan-Meier); adjusting for baseline covariates, HR = 0.59 (0.42, 0.82) for RD vs rd, *P* = .002 (Cox regression). Patients receiving rd had a lower incidence of grade 3/4 AEs vs RD, including neutropenia (27% vs 41%), thrombocytopenia (7% vs 16%), and thrombotic events (3% vs 13%). Lenalidomide dose reductions were 34% vs 46% and dexamethasone dose reductions were 41% vs 31% for rd and RD, respectively. Duration of treatment was shorter with rd vs RD (7.3 vs 9.2 months). Similar efficacy and safety outcomes were observed for patients with CrCl ≤ 60 mL/min. **Conclusions**. In patients who are elderly and/or have renal impairment or thrombocytopenia, rd is a reasonable option with improved tolerability. In view of the potential survival advantage associated with the full dose regimen, risk-benefit should be considered when choosing the dose of lenalidomide and steroids. Standard-risk patients should receive the approved RD dosing demonstrated to improve PFS and OS in two large randomized studies.

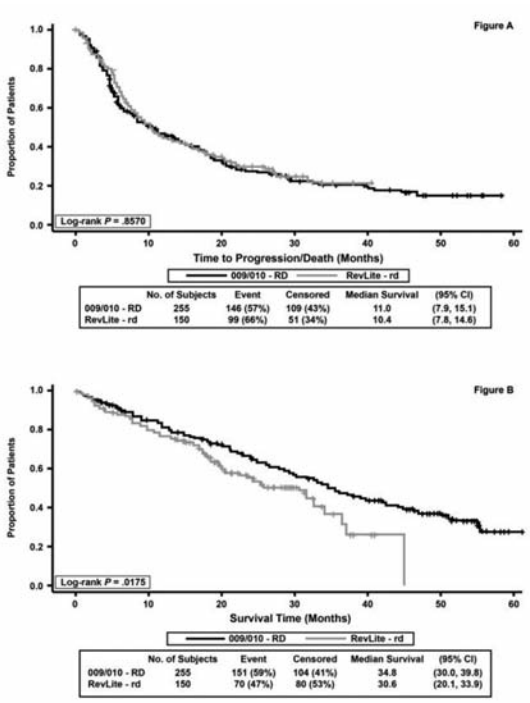

Figure 1. Progression-free and overall survival for patients from RevLite and MM-009/010 trials

0855

SEQUENTIAL HIGH-DOSE DEXAMETHASONE AND RESPONSE ADAPTED PAD OR VAD INDUCTION CHEMOTHERAPY FOLLOWED BY ASCT FOR NEWLY DIAGNOSED MM; MULTICENTER PHASE 2 STUDY (KMM-94 STUDY)-INTERIM ANALYSIS

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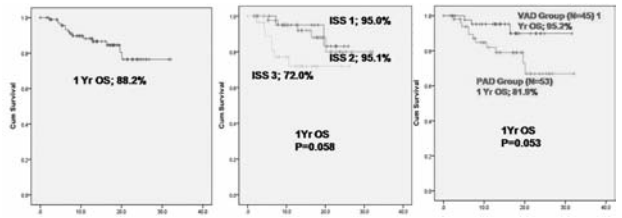
Background. Induction treatment followed by ASCT is the standard therapy for the newly diagnosed younger patients with MM. Although new drugs such as lenalidomide or bortezomib have been shown the promising results as induction treatment, many different type of induction treatment regimens still have been used. We evaluate the efficacy and safety of the short course of high dose dexamethasone (HD dexa) and the response adapted PAD (Bortezomib, Adriamycin, Dexamethasone) or VAD induction chemotherapy in the newly diagnosed younger patients with MM. **Methods.** 125 newly diagnosed patients with MM from 21 institutions received 2nd cycles of HD dexa followed by PAD or VAD chemotherapy according to the response to the initial high dose dexamethasone. The primary endpoint was complete response (CR) + near CR rate after ASCT. Among 125 patents enrolled this study from November 2009, 27 patients (22%) have been dropped out. This trial will be continued until total 210 patients will be enrolled. The trial is registered on National Cancer Institute website, number NCT01255514. **Results**. 125 patients (67 male, 58 female) were enrolled (median age; 56). 30 (24%) light chain disease were included. 34 (27%) patients were D-S stage II and 82 (66%) were stage III. According to the ISS, 27 (22%) patients had stage I, 59 (47%) had stage II and 39 (31%) had stage III. 26% patients had abnormal cytogenetics. There were 29% del13, 7% del17, 19% t(4;14), 14% t(14;16) and 25% t(11;14) in FISH analysis. Among the 98 evaluable patients, CR + PR rate was 46% (45/98) after 2nd cycles of HD dexa therapy. 45 patients (46%) received subsequent VAD chemotherapy and 53 patients (54%) received PAD chemotherapy. Among the 85 patients finished VAD or PAD chemotherapy, CR + PR rate was 80% (79%, 32/41 in VAD group vs. 80%, 36/44 in PAD group). 75 patients were finished ASCT until now. CR + near CR rate after ASCT were 75% (71% in VAD group vs. 80% in PAD group). Mortality rate during the treatment was 7% (7/98). The cause of death was disease progression (n=1), bleeding (n=1) and infections (n=5). Among 98 patients in whom VAD or PAD chemotherapy was actually performed, 1 year overall survival (OS) rate was 89.2%. 1 year survival rate was 95.2% vs. 81.9% (P=0.052) with VAD vs. PAD (median follow-up; 13.7 months). **Conclusions**. Risk adapted approach using initial steroid response showed good response results after ASCT compared with previous trial (CR + near CR rate of IFM 2005-01 trial-Bortezomib+dexa induction & ASCT was 35%, J Clin Oncol. 2010;28:4621-9) The MM patients who had poor response to HD dexa also showed similar good response rate after ASCT compared with the patients

who had good response to HD dexa treatment in this trial. PAD re-induction therapy after failure of initial steroid induction treatment might overcome the inferior results in the high risk MM patients. Therefore, initial steroid response adapted strategy might be the more cost-effective and might reduce the burden of induction therapy for lower-risk newly diagnosed ASCT eligible MM patients.



0856

BOTH HEMATOLOGIC AND RENAL RESPONSE AFFECT OVERALL SURVIVAL OF MYELOMA PATIENTS WITH ACUTE KIDNEY INJURY

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Background. Acute kidney injury (AKI) is a common but serious sequela of multiple myeloma. Recently, the International Myeloma Working Group (IMWG) introduced a new renal response criteria with complete (CRenal), partial (PRenal) and minimal (MRenal) responses. This study compares the IMWG criteria with other models to determine their correlation with overall survival (OS). **Methods.** Patients with AKI (serum creatinine (Scr) ≥2.0 mg/dl) complicating multiple myeloma were identified from international centers. OS was calculated from the day of AKI. Renal function was assessed by eGFR using the MDRD method. Hematologic response was the best achieved as assessed by International Uniform Response Criteria for Multiple Myeloma. Separate analyses were performed on the newly diagnosed (ND) patients versus previously treated (PT) patients.

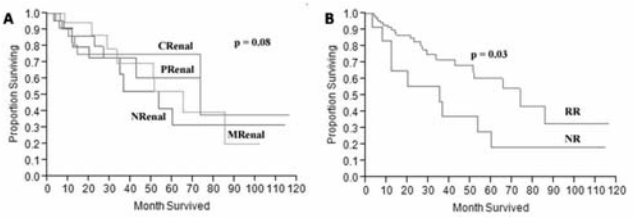

Figure 1. (A) Overall survival (OS) of 115 patients evaluated by the IMWG renal response criteria. OS was not significantly different by Log-Rank test between patients with CRenal and NRenal, p = 0.08. (B) In a separate model, 85 newly diagnosed patients with hematologic response of PR or better were evaluated. Patients who were dialysis independent achieved a renal response (RR) and those who remained on dialysis had no response (NR). Patients with RR had a superior overall survival than patients with NR. Renal response was independent of a hematologic response of PR or better.

Results. A total of 143 patients were collected of these 115 were ND, median age was 64 (34-87) years and 58.7% were male. Median Scr was 5.1 (2.0 to 18.6) mg/dl and eGFR was 10.6 (2.8-36.5) ml/min/1.72m^2. Dialysis was required in 49.3%, and 72.9% were dialysis independent at the end of the study. By IMWG criteria, median OS ranged between 6.0 months (m) in PT patients with no renal response (NRenal) to 23.7m in patients with CRenal, p=0.34. In ND patients, OS was 31.4m in NRenal vs 73.8m in CRenal (p=0.08). However, the difference in OS between PRenal and CRenal was not significant, p=0.70 (Figure 1A). If only patients with a hematologic PR or better were analyzed, the OS range was 6.0m in NRenal to 23.7m in CRenal in PT patients (p=0.85) and 36.6m in NRenal to 73.8m in CRenal in ND patients, p=0.73.The best model tested was one that defines renal response (RR) as regaining or maintaining dialysis independence and no response (NR) as dialysis dependence. Patients who achieved RR had a superior survival than NR for both PT patients (13.5m vs 6.0m respectively, p=0.03) and ND patients (73.8m vs 20.1m respectively, p <0.001). RR increased with the depth of hematologic response, p = 0.04. RR occurred in 75% of the PT patients with hematologic PR or better and 66.7% in those with less than PR, p=0.64. In ND patients, RR occurred in 85.7% of those with PR or better and 54.6% of those with PR or less, p=0.003. This may explain the differences in OS when hematologic response was considered. In PT patients with hematologic PR or better, renal response did not improve OS (21m vs 6m, p=0.28) but it remained beneficial in the ND patients (73.8m vs 35.1m, p=0.04, Figure 1B). Renal response was no longer significantly associated with OS for any patient with hematologic response that was VGPR or better. In a multivariate analysis, RR (p=0.001), ND (p <0.001) and PR or better hematologic response (p=0.046) were associated with OS. **Conclusions.** The results of our study suggest renal response is important to OS but the depth of response was not as important. Based on our results, reducing the number of response categories and simplifying the current consensus criteria seems justified.

0857

CARFILZOMIB IS ASSOCIATED WITH A LOW RATE OF TYPICALLY MILD TO MODERATE, NON-DOSE LIMITING TREATMENT-EMERGENT PERIPHERAL NEUROPATHY

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Background. Patients with multiple myeloma (MM) often experience debilitating peripheral neuropathy (PN) as part of their disease progression, and many drugs used to treat MM can worsen or cause painful, treatment-limiting PN. Identifying next-generation agents that demonstrate minimal treatment-emergent PN is critical. Carfilzomib, a proteasome inhibitor being evaluated for the treatment of relapsed and/or refractory MM, has demonstrated less neurodegeneration than bortezomib in pre-clinical models, and early clinical studies of carfilzomib suggest a favorable PN profile. **Aims**. To further assess treatment-emergent PN following therapy with single-agent carfilzomib, a cross-trial analysis of 526 patients from 4 phase 2 studies, many of whom had baseline PN or a history of PN, has been performed and the results provided herein. **Methods.** Patients receiving carfilzomib in the following trials are included in this analysis: PX-171-003-A0, PX-171-003-A1, PX-171-004, and PX-171-005. In all studies, carfilzomib was dosed on Days (D) 1, 2, 8, 9, 15, and 16 of a 28-day cycle (C). Doses were 20 mg/m^2 in C1, escalating to 27 mg/m^2 in C2 as defined per individual protocol for all studies except 005 (C1, C2, C3 were 15, 20, 27 mg/m^2 respectively). Assessments for these patients included neuropathy history and baseline neuropathy status, along with directed neurologic physical exams and FACT/GOG-Ntx v 4.0 questionnaires at screening and on D1 of Cycles 3, 5, 7, 9, and 11, and at the end of the study. Assessment of PN was based on an aggregate of PN terms, including peripheral neuropathy, neuropathy, peripheral sensory neuropathy, and peripheral motor neuropathy. **Results**. The majority of patients (84.8%) in these studies had medical histories significant for PN in prior treatments (42.6% due to bortezomib and 43.3% due to thalidomide, which resulted in discontinuation of those treatments in 25.9% and 21.1% of patients, respectively). During treatment with carfilzomib, PN occurred infrequently (13.9% overall) across all studies. Of 526 patients, 41 (7.8 %) experienced Grade 1 PN, 25 (4.8 %) Grade 2 PN, and 7 (1.3%) Grade 3 PN. Notably, no ≥Grade 4 PN was reported. In response to a PN-related AE, 1 (0.2%) patient discontinued treatment and 4 (0.8%) required a dose modification. Although 378 (71.9%) patients had active PN at baseline (all Grade 1 or Grade 2), the majority (87.3% [330/378]) did not report AEs related to PN at any time on treatment or within 30 days of last dose. **Conclusions**. In contrast to currently approved MM therapies,carfilzomib is associated with a low rate of mild to moderate, non-dose-limiting PN, causes a low rate of treatment-emergent PN in patients with a history of PN, and does not exacerbate PN in patients with pre-existing neuropathy. Dose modifications and discontinuations due to PN are extremely low, allowing for longer duration of treatment for MM.

0858

BENDAMUSTINE, BORTEZOMIB AND DEXAMETHASONE (BVD) COMBINATION FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA: AN INTERIM ANALYSIS OF A PHASE II STUDY

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Background. Bendamustine is a bifunctional purine analog/alkilator hybrid compound having no-cross resistance to other alkylating agents and that proved to be effective either in relapsed/refractory and in naïve MM as single agent or combined with steroids. In vitro, Bendamustine showed additive/synergistic activity with bortezomib which in turn synergizes with dexamethasone. **Aims.** The primary objective of this phase II, multicenter study was to assess the activity (response rate ≥ PR after 4 cycles) and the toxicity of the BVD combination. Secondary end-points were best response at the end of therapy, PFS and OS. **Methods.** Patients pre-treated with no more than 4 regimens, not refractory to bortezomib and with adequate laboratory tests, received Bendamustine 70 mg/m^2 on days 1 and 8, Bortezomib 1.3 mg/m^2 on days 1, 4, 8, 11 in the first 2 cycles and 1.3 mg/m^2 on day 1, 8, 15, 22 thereafter, Dexamethasone 20 mg the same day and the day after bortezomib every 28 days for 4 cycles of induction. Patients who obtained less than PR went off-study whereas those achieving at least PR after induction received 2 additional cycles every 28 days and thereafter other 6 cycles every 56 days as consolidation. Antimicrobial prophylaxis with acyclovir and thrimethoprim-sulfamethossazole was mandatory while G-CSF and EPO could be administered on demand. Response and toxicity were assessed according to IMWG and CTCAE version 3 criteria, respectively. According to Fleming method, 70 patients would have to be recruited to demonstrate a PR rate of 60% with 80% of power and 5% of type 1 error. This is a not pre-planned interim analysis. **Results.** As February 25, 2012, a total of 44 patients were enrolled in 11 Italian Centres. Thirty patients completed at least one cycles and are evaluable for response and toxicity. Median age was 69.5 years (range 53-83) and 11 patients (36%) had PS (WHO) = 2. Twenty-two patients had ISS stage ≥ 2, 8 (27%) had renal insufficiency and 10 (33%) had received ≥ 2 prior regimens. All patients had been just treated with regimens containing new-drugs (50% thalidomide, 57% lenalidomide, 40% bortezomib, 40% two new-drugs) and 50% had received alkylators. Seven patients underwent one cycle, 4 two cycles, 3 three cycles and 16 four cycles. At least PR was obtained in 23 patients (77%). Five patients (17%) achieved CR, 6 (20%) VGPR and 12 (40%) PR and 3 (10%) SD whereas 4 (13%) progressed. Grade 3-4 adverse events comprised 30% thrombocytopenia, 17% anemia, 10% neutropenia, 13% infections. Only 1 patient developed grade 3 peripheral neuropathy; another 2 had severe cardiac and liver toxicity, respectively. There were 2 early deaths due to pneumonia with septic shock and 1 sudden death during 2nd cycle. Only one patient discontinued therapy because of toxicity and 4 patients (13%) reduced therapy mainly due to hematologic toxicity. **Conclusions.** BVD combination shows a remarkable anti-myeloma activity in patients previously treated with any new-drugs with manageable hematological and non-hematological toxicity. Pneumonia represented the main, sometimes fatal, complication warranted adequate prophylaxis.

0859

EUROPEAN POST-APPROVAL SAFETY STUDY (EU PASS) OF LENALIDOMIDE COMPARED WITH OTHER TREATMENTS IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: THE ITALIAN 'REAL-WORLD' EXPERIENCE

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Background. Outcomes of patients in clinical trials often differ from those of patients in daily clinical practice. The safety and tolerability of novel treatments for multiple myeloma (MM) in the "real-world"setting are important considerations, but have been insufficiently investigated. **Aims.** To evaluate the tolerability of lenalidomide, bortezomib, and thalidomide as salvage therapy for patients with relapsed or refractory MM (RRMM). **Methods.** This multicenter, observational, non-interventional post-authorization safety study included patients with RRMM who had received at least one prior therapy and were commencing a new treatment. Patients were enrolled into the lenalidomide cohort (lenalidomide plus dexamethasone, the approved combination for the treatment of RRMM) or background. cohort (all other treatments, including other novel agents) at the investigator's discretion. Thromboprophylaxis was allowed, but not mandated. Assessments for second primary malignancies (SPM) were to be conducted up to 36 months after treatment discontinuation. **Results.** As of November 2011, 768 RRMM patients were enrolled at 54 institutions in Italy. Of these, 510 received lenalidomide, 219 bortezomib, 13 thalidomide, and 26 other therapies. In the background. cohort, 26 patients crossed over to lenalidomide. The median follow-up was 25 weeks (range, 0.6-117.4), the median age was 70years (range, 40-95), and 53% of patients were male. Most patients had a good performance status (ECOG 0-1), while 22.1% had an ECOG score of 2-4. All patients were heavily pre-treated; overall, the median number of prior treatments was 2 (range, 0-6), 33% had received 2 prior treatments and 19% had received ³3; 35% had undergone autologous stem cell transplantation. In the lenalidomide group, 59% of patients received ³2 prior treatments, while 35% of bortezomib-treated patients and 31% of thalidomide-treated patients had ³2 prior regimens. Baseline characteristics were similar across groups. The incidence of grade 3-4 and serious AEs is reported in the Table. Notably, the incidence of end-stage renal disease (ESRD) was similar in the lenalidomide and bortezomib groups (5.1% and 4.1%, respectively); 2 thalidomide-treated patients had ESRD. Venous thromboembolism (VTE) occurred in 5% of lenalidomide-treated patients (2% grade 3-4) and 1% of bortezomib-treated patients. Peripheral neuropathy (PN; all grades) was observed in 28% of bortezomib-treated patients and 10% of lenalidomide-treated patients; 4% and 2% grade 3-4, respectively. In total, 61% of patients discontinued lenalidomide therapy, while 80% and 77% of patients discontinued bortezomib and thalidomide, respectively. Primary reasons for treatment discontinuation were overall AEs and disease progression. Incidence of SPM was very low (0.1%), with 1 case of leukemia in the lenalidomide group. Overall, patients had favourable prognoses; only 1% of deaths due to AEs were thought to be treatment-related (lenalidomide, 1%; bortezomib, 1%; thalidomide, 0%). **Conclusions.** Lenalidomide plus dexamethasone is associated with survival benefit in clinical trials of RRMM. In this "real-world"study, ESRD did not impact ini-

tial treatment choice. Incidence of AEs was similar across treatment groups, with the exception of higher rates of PN and VTE in bortezomib and lenalidomide-treated patients respectively. Only one SPM has been observed to date.

Table. Incidence of adverse events (%).

AEs, (%)	Lenalidomide (n = 510)	Bortezomib (n = 219)	Thalidomide (n = 13)
Any grade 3–4 AE	44	32	23
Drug-related grade 3–4 AE	30	23	8
Drug-related serious AE	10	3	8
AE leading to death	7	5	0
All-grade VTE	5	1	0
Grade 3–4	2	0	0
All-grade PN	10	28	0
Grade 3–4	2	4	0
Discontinuation	61	80	77
Due to AEs	10	12	15
Due to progressive disease	18	12	31

AE, adverse event; PN, peripheral neuropathy; VTE, venous thromboembolism.

0860

FLOW CYTOMETRY AS A PREDICTIVE TOOL FOR TRANSFORMATION OF MGUS INTO MM

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Background. Monoclonal gammopathy of undetermined significance (MGUS) is a premalignant state with possibility of the transformation into multiple myeloma (MM). Traditional prognostic parameters have limited sensitivity and specificity to predict transformation within 3 years from time of diagnosis. MGUS is characterised by presence of mixture of normal $CD19^+$ and abnormal $CD19^-$ plasma cells (PCs). Disappearance of $CD19^+$ PCs should correlate with transformation, but there are other markers exist which could be useful in prediction of transformation MGUS into MM. **Aims.** Flow cytometry analysis of surface antigens and their comparison with standard prognostic factors to predict possibility of transformation MGUS into MM. **Methods.** Analysis of 150 untreated MGUS cases in time of diagnosis was done. Number of $CD19^+$ B cells and $CD38^+CD138^+$ PCs was analysed in whole bone marrow. Expression of CD19, CD56, CD20, CD27, CD28 and CD117 on PCs was analysed by flow cytometry. Parameter $CD19^-$ PCs is equivalent to abnormal PCs. Flow cytometry results were compared with standard prognostic factors. **Results.** Transformation of MGUS into MM was found only in 9 subjects. These have statistically decreased number of B cell [7.0% (0.0-23.7) vs. 13.3% (1.2-68.4); p<0.02], increased number of PCs [1.0% (0.2-8.8) vs. 0.4% (0.0-4.2); p<0.01] and decreased number of normal $CD19^+$ PC [3.8% (0.9-33.4) vs. 25.9% (0.2-87.2); p<0.002] when compared to subjects without transformation. There were found non-significant decrease of CD27 and increase of CD56 expression on PCs in transformed group. Surprisingly, there was found almost IgG isotype of clonal immunoglobulin (MIG) in 93.5% (29/32) subjects with $CD28^+$ PCs. Significantly higher MIG concentration (>15g/l) was found only in cases with $CD19^+$ PC ≤5% (p=0.001), but abnormal FLC index was found already when $CD19^+$ PC <20% (p=0,017). Subjects with $CD19^+$ PC ≤5% were more frequently presented in high medium risk group when compared to subjects with $CD19^+$ PC >5% presented more frequently in low medium risk group (p=0.009). When analysed connection between parameter "$CD19^+$ PC ≤5%" and progression into MM, this parameter was able to predict progression with 55.6% sensitivity and 91.2% specificity (p=0.019). **Conclusions.** Flow cytometry in analysis of MGUS cases seems to be helpful approach. The most powerful factor is presence and/or loss of CD19 on PCs. Decrease of relative count of $CD19^+$ PC under 5% (from whole PC population) can predict possibility of transformation into MM what is in concordance with previously published results.
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0861

BORTEZOMIB-BASED THERAPY INCREASES THE INCIDENCE OF POST-TRANSPLANT VIRAL INFECTIONS IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTO-SCT: A RETROSPECTIVE ANALYSIS FROM THE ROME TRANSPLANT NETWORK

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Background. The recent large use of proteasome inhibitors (i.e. Bortezomib) and immunomodulator agents (i.e. Thalidomide and Lenalidomide) in pre-transplant treatment has determined an increase in the global incidence of viral infections in MM patients. On the contrary, multiple myeloma (MM) patients undergoing conventional chemotherapy with VAD (Vincristine, Doxorubicin and Dexamethasone) followed by autologous hematopoietic stem cell transplantation (AutoSCT) have a relatively low risk of post-engraftment viral infection by herpesviridae family viruses (Cytomegalovirus, CMV; Varicella-Zoster Virus, VZV; Herpes Simplex Virus 1 and 2, HSV1-2). **Aims**. The aims of the present study were: 1) to establish the post-engraftment incidence of viral infection in MM patients undergoing AutoSCT after treatment with proteasome inhibitors and immunomodulator agents; 2) to perform a retrospective comparison with patients treated with only VAD regimen before AutoSCT. **Methods.** We performed a retrospective analysis on 80 consecutive adult patients (median age 54 years, range 38-69) with a diagnosis of MM who underwent AutoSCT following treatment with proteasome inhibitors and/or immunomodulator agents in the Hematology Institutions participating in the Rome Transplant Network (RTN) from January 2008 to July 2010 (Group A). Eighty-nine MM patients with similar characteristics who underwent AutoSCT in the RTN after treatment with conventional chemotherapy from January 2004 to January 2008 represented the control group (Group B). **Results.** In group A, 53/80 patients (66.2%) were treated with Bortezomib (with or without immunomodulator agents) and 27/80 (33.8%) with only immunomodulators agents. Overall, in this group, 9 patients received a specific antiviral therapy for a viral infection after AutoSCT (CMV n=6, VZV n=2, HSV n=1) with a global incidence of 11.3%. Of these 9 patients, 6 were treated with a specific antiviral therapy for a symptomatic CMV infection. No cases of end-organ disease according to published criteria were observed. The global incidence of viral infections and CMV symptomatic infections was significantly higher in group A than in group B. In particular, viral infections incidence was 11.3% vs 2.2% (P=0.026) and CMV symptomatic infection incidence was 7.5% vs 1.1% (P=0.05) in group A and B respectively. Transplant-related mortality (TRM), neutrophils and platelets engraftment and clinical outcome were similar in the two groups. Considering separately the 53 patients who received a Bortezomib-based regimen prior to AutoSCT, the incidence of viral infections and CMV symptomatic infections was significantly higher when compared with that of patients not given Bortezomib before AutoSCT. In particular, viral infection incidence was 13.2% in the Bortezomib group vs 3.4% in the remaining patients (P=0.037) and CMV symptomatic infection incidence was 9.4% in the Bortezomib group vs 1.7% in the remaining patients (P=0.032). **Conclusions.** From our retrospective analysis, MM patients treated with novel agents and in particular with Bortezomib-based therapy seem to be at higher risk to develop clinically relevant herpesviridae family infections, in particular from CMV, after AutoSCT compared with patients treated with conventional pre-transplant chemotherapy. A post-AutoSCT monitoring for viral infection could be recommended in these patients in order to start promptly a specific antiviral therapy.

0862

HIGH DOSE THERAPY AND ASCT AS CONSOLIDATION AFTER SECOND LINE TREATMENT OF MULTIPLE MYELOMA RELAPSED AFTER FIRST LINE ASCT: ANALYSIS OF PROGNOSTIC FACTORS

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Background. Baseline clinical and laboratory findings have been identified as prognostic factors and for risk stratification of newly diagnosed pts. Few studies have been performed exploring this aspects at relapse. **Aims**. To analyse impact of characteristics at first relapse on PFS after second line therapy of a consecutive series of relapsed MM pts receiving up-front ASCT. **Methods** .In 217 MM pts treated at our Institution between 1997 and 2009 with first-line HD-Mel and ASCT, relapse occurred in 160 pts (74%).As induction therapy before ASCT, 177 (82%) had received VAD and 40 (18%) thalidomide (T)/bortezomib **-based regimens. Ninetyfive (44%) had received double ASCT. Second-line therapy was given to 137 (86%) pts: in 94 (69%) with** based regimens only, in 43 (31%) with ASCT as consolidation after TB-regimens (n 28) or chemotherapy (n 15). Median follow-up from diagnosis was 60 mo (12-168). CR/VGPR and ORR after first line therapy was 59% and 94%.The following characteristics at relapse were considered potential prognostic factors: gender, age (>65 vs <65), serum and urine monoclonal component, bone marrow plasmocytosis, ISS (I vs II-III), bone progression, haemoglobin (>10 g/dl vs < 10 g/dl) and creatinine (> 2 mg/dl vs < 2 mg/dl) level, hypercalcemia, response after second-line therapy (>VGPR), type of second line therapy (ASCT as consolidation vs TB-regimens only), time of relapse after first line therapy (> 12mo vs < 12mo). **Results**. CR/VGPR and ORR (CR+VGPR+PR) after second line therapy were 27% and 64% respectively. After a median follow-up from second line therapy of 51 months (11-168 mo), 2 yrs PFS was 20% (median 15 mo) and 2 yrs OS 69% (median 37 mo).CR/VGPR rate and PFS was significantly better after ASCT consolidation than after TB regimens only (CR/VGPR 47% vs 18%, median PFS 16 vs 11 mo- p 0.036).At univariate analysis female sex, low haemoglobin level, hypercalcemia, high ISS, relapse < 12mo after first line therapy, < VGPR after second line therapy and TB regimens adversely affected the duration of PFS. At multivariate analysis high ISS and < VGPR after salvage therapy retained independent adverse prognostic value (p 0.002). **Conclusions**. Our study confirm ISS at relapse and the quality of response to therapy as important prognostic factors also in the relapse setting. ASCT after second-line treatment increased CR/VGPR rates and significantly impacts on PFS. It should be considered a valid therapeutic adjunct in patients who do not achieve a VGPR after second-line TB regimens.

0863

AMONG MICROENVIRONMENTAL CYTOKINES WITH BIOLOGIC ACTIVITY IN MULTIPLE MYELOMA (MM), ONLY SERUM SOLUBLE SYNDECAN-1 CORRELATES WITH FLCR; SUGGESTED ROLE IN FLC SECRETION CONTROL

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Background. The mechanisms of FLC inappropriate over-secretion by malignant plasma cells are unknown and probably relate to genetic and/or microenvironmental factors. **Aims.** To study any possible correlation between multiple myeloma (MM) patients' serum FLC and FLCR levels and serum cytokines with a well-known involvement in MM biology, namely soluble syndecan-1 (s-synd-1), IL-6R, VEGF, bFGF, TGF-beta, IGF, MIP-1alpha, OPG, RANKL, MMP-2, MMP-9, and BLyS. **Patients and Methods.** In 181 MM patients with available frozen serum aliquot collected at diagnosis, serum cytokine levels were retrospectively determined by ELISA KITs according to the manufacturers' instructions; resulted values were correlated with baseline serum FLC and FLCR. Serum FLCs were measured by nephelometry (FREELITE, The Binding Site Ltd. Birmingham, UK). The light chain ratio (sFLCR) κ/λ or λ/κ was built with the uninvolved LC as denominator. Statistical analysis was performed by standard **Methods.** Differences were considered of statistical significance when p values were below 0.05. **Results.** Serum s-synd-1 levels, determined in 155/181 ranged from 7 to 5070 ng/ml (median, 85 ng/ml), IL-6R (in 91) from undetectable to 43301 pg/ml (median, 5949 pg/ml), VEGF (in 100) from undetectable to 4000 pg/ml (median, 210 pg/ml), bFGF (in 66) from 3 to 60 pg/ml (median, 10 pg/ml), TGF-beta (in 42) from 1350 to 99000 pg/ml (median, 61500 pg/ml), IGF (in 61) from 13 to 260 pg/ml (median, 84 pg/ml), MIP-1alpha (in 97) from 14 to 100 pg/ml (median, 32 pg/ml), OPG (in 87) from 820 to 25000 pg/ml (median, 3100 pg/ml), RANKL (in 59) from 0.08 to 10 pmol/L (median, 0.19 pmol/L), MMP-2 (in 57) from 84 to 700 pg/ml (median,250 pg/ml), MMP-9 (in 69) from 53 to 1000 pg/ml (median, 230 pg/ml), and BLyS (in 102) from undetectable to 3482 pg/ml (median, 155,5 pg/ml). Involved serum FLC and FLCR values strongly correlated with s-synd-1 (p=0.008 and <0.0001 respectively) while no statistical significant correlation was found for all other cytokines studied although some of them shared with FLC, FLCR and s-synd-1, prognostic information. Likewise an inferior overall survival was observed in patients with FLC (p=0.001), FLCR (p<0001), s-synd-1 (p<0001), sIL-6R (p=0.02), BLyS (p=0.04), IGF-1 (p=0.04, inversed), RANKL (p=0.006, inversed), MMP-2 (p=0.001) serum levels above median. **Conclusions**. The strong relationship between s-synd-1 and FLC or FLCR, that was not observed with the other cytokines studied, suggest a biological involvement of syndecan-1 in FLC secretion's modulation.

0864

IN PATIENTS WITH MYELOMA AND RENAL FAILURE FREE LIGHT CHAIN LEVELS (SFLC) CAN BE REDUCED GREATLY WITHIN 2 WEEKS AND LOWER SFLC LEVELS ARE ASSOCIATED WITH A BETTER CHANCE OF RENAL RECOVERY

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Background. In patients presenting with myeloma and renal failure the aetiology of renal damage is multi-factorial but serum immunoglobulin free light chains (sflc) from the malignant clone are the predominant cause. Particularly in patients on dialysis sflc measurements are superior to urine flc measurements in determining the flc load that the kidney is exposed to. Since 2002 it has been possible to quantitate the levels of sflc in routine clinical practice. **Aims.** In a prospective study we measured levels of sflc at diagnosis and serially thereafter to determine if lower sflc levels are associated with a higher chance of renal recovery. Accordingly we attempted to maximise rapid reduction of sflc and to measure that reduction. All patients received dexamethasone alone for two weeks from entry to the trial. Additionally they were randomised to receive 7 plasma exchanges or not. **Methods.** The UK MERIT trial recruited 78 newly diagnosed myeloma patients with acute renal failure unresponsive to treatment with fluid and/or treatment of hypercalcaemia with bisphosphonate (creatinine >500mmol/l, urine output <400 ml/d or requiring dialysis). 40 patients were randomised to no plasma exchange (PE) and 38 to PE; 7 plasma exchange treatments by cytocentrifugation or plasma filtration (days 1-14; 4 in days 1-7). All patients received dexamethasone 40 mg days 1-4 and 9-12 and from day 17-100 chemotherapy as specified at randomisation by the treating clinician. The primary end point was the proportion of patients alive and dialysis-independent at 100 days. Serum flc levels (mg/l) were measured centrally on days 0, 5, 10, and 17 and these levels compared between the 22 patients who were alive and dialysis free at day 100 and the 56 patients who were not. **Results.** In the PE group at 100 days 11 (28.9%) patients were alive and dialysis Independent, 17 (44.7%) were alive and dialysis dependent, 7 (18.4%) were dead and 3 (7.9%) missing data.In the No PE group at 100 days 11 (27.5%) patients were alive and dialysis Independent, 21 (52.5%) were alive and dialysis dependent, 5 (12.5%) were dead and 3 (7.5%) missing data.There is no evidence of a difference between these two treatment groups (p=0.876).At entry median sflc levels were 6,319 (range 800-57,695) and the median for lowest sflc level in the first 2 weeks was 1655 (191-32767) with no difference between treatment arms. Results show that for every percentage reduction in log-FLC from baseline, the odds of being alive and dialysis independent are 6.6% greater (p-value=0.015). **Conclusions.** Lower levels of serum flc at diagnosis and greater percentage reductions in those levels are associated with improved chance of renal recovery. PE removes large quantities of sflc but across the trial this was insignificant compared to the reduction achieved by dexamethasone. Dexamethasone can achieve rapid hundred fold reductions of flc levels but response is very variable between patients and should be monitored at early intervals to allow consideration of a change of therapy.

0865

HIGH LEVELS OF NEURAL STEM CELL MARKER NESTIN ARE ASSOCIATED WITH 1Q21 GAIN AND PREDICT WORSE RESPONSE TO CONVENTIONAL AND NOVEL THERAPY IN MULTIPLE MYELOMA

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Backround. Nestin, an intermediate filament protein, is detected in undifferentiated multipotent cells of some embryonic and fetal tissues. During terminal differentiation, nestin expression is diminished but may be reexpressed under certain pathological conditions, such as injury or cancer. Nestin is a suitable diagnostic and prognostic indicator of malignancy and potential cancer stem cells marker in solid tumors. Our recent work confirmed nestin protein as a tumor-specific marker for $CD138^+38^+$ plasma cells (PC) and proved significant higher nestin levels in multiple myeloma (MM) compared with controls without any hematological malignancy. **Aims.** The aim of this study was i) to analyze relationship between nestin expression and cytogenetic aberrations in $CD138^+$PC, ii) to analyze relationship between nestin and standard prognostic parameters and iii) evaluate whether pretreatment levels of nestin predict response to therapy. **Methods.** A total number of 93 newly diagnosed MM patients (36M/47F; median age 70 years) were enrolled in this study. Gene expression of nestin was analyzed by quantitative real-time PCR in 70.9% (66/93) of MM patients and quantified by the comparative ddCt method. Nestin was detected in $CD138^+38^+$PC of 50.5% (47/93) of MM patients and 10 individuals without hematological malignancy by flow cytometry. Nestin levels were assessed as the percentage of nestin-positive PC (%Nes^+PC) and ratio of median fluorescence intensity of nestin (MFI) and isotypic control. $CD138^+$PC of MM patients were analyzed for del(13q14), del(17p53), IgH rearrangement, 1q21 gain and hyperdiploidy by FISH. Pretreatment levels of nestin mRNA and protein were evaluated in MM patients treated with conventional therapy or novel agents without autologous stem cell transplantation. Differences among groups were analyzed by non-parametric Mann-Whitney U test or Kruskall Wallis H test. Correlation was analyzed by Spearman correlation coefficient. **Results.** Significant differences between MM patients and the control group were demonstrated based on %Nes^+PC (98.5 vs. 0.1; $p<0.00001$) and MFI ratio (3.5 vs. 2.0; $p<0.0001$). High %Nes^+PC ($p=0.007$) and MFI ratio ($p=0.012$) were significantly associated with 1q21 gain-positive patients. No correlation between gene or protein expression of nestin and standard prognostic parametres was confirmed. Differences of MFI ratio between patients who achieved at least VGPR and patients with worse response reached only borderline significance ($p=0.053$). Patients who achieved ≥VGPR had lower nestin expression compared with patients who achieved ≤PR [median RQ 1.3(0.6-3.9) vs median RQ 4.6(1.7-152.0); $p=0.001$]. **Conclusions**. MM patients have significantly higher levels of nestin protein and percentage of Nes^+PC compared with the control group. The association with 1q21 gain may be explained by the fact that nestin gene is located at 1q23.1 and gain of whole q-arm of chromosome 1 is found in most MM patients. Relationship between nestin and other prognostic parameters was not proved. High levels of nestin mRNA appear to be a potential predictor of worse response in patients treated with conventional therapy or novel agents. This pilot study needs to be further validated on a larger cohort of patients.

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0866

EUROPEAN POST-APPROVAL SAFETY STUDY (EU PASS) COMPARING LENALIDOMIDE WITH OTHER TREATMENTS IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: THE SPANISH EXPERIENCE

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Background. Lenalidomide is an effective antimyeloma therapy that is currently approved in combination with dexamethasone by the US Food and Drug Administration and the European Medicines Agency, as well as in other countries for the treatment of multiple myeloma (MM) patients who have received at least one prior therapy. Although many studies have described the safety of antimyeloma drugs in clinical trials, few have evaluated the tolerability of these agents in daily clinical practice. **Aims.** To characterize the safety profile of lenalidomide and compare the incidence of adverse events (AEs) in patients treated with lenalidomide versus other antimyeloma therapies in daily clinical practice in Spain. **Methods.** This multicenter, observational, non-interventional, post-authorization safety study included patients with relapsed or refractory MM (RRMM) who had received at least one prior therapy and were commencing a new treatment. Patients were enrolled into the lenalidomide cohort (lenalidomide plus dexamethasone, the approved combination for treatment of RRMM) or the background. cohort (all other treatments, including novel agents) at the investigator's discretion. Thromboprophylaxis was allowed but not required.

Table. Incidence of adverse events (AEs) according to NCI-CTCAE (version 3) grading (%).

AEs, (%)	Lenalidomide (n = 323)	Bortezomib (n = 107)	Other, including thalidomide (n = 27)
Any grade 3–4 AE	55	48	44
Drug-related grade 3–4 AE	39	22	19
Drug-related serious AE	16	11	0
All-grade hematologic AE	51	40	44
Grade 3–4			
Neutropenia	24	5	11
Thrombo-cytopenia	14	13	7
Anemia	12	11	4
Drug-related AE leading to death	3	2	0
All-grade VTE	2	0	0
Grade 3–4	1	0	0
All-grade PN	3	23	0
Grade 3–4	1	3	0

Results. As of November 2011, 457 RRMM patients were enrolled in Spain. Of those, 323 received lenalidomide, 107 bortezomib, and 27 other therapies, including 5 patients who received thalidomide or had missing data. Twenty-two patients from the background. cohort crossed over to receive lenalidomide. Baseline characteristics were similar across cohorts. The median age was 71 years (range, 34-90); 53% were female. The median number of prior therapies was 2 (range, 0-6); 70% had received 2 prior therapies and 20% had received 3 or more. Overall, 22% had received prior stem cell transplantation. The median follow-up for all groups was 5.4 months (range, 0.3-28.1). Prior to treatment initiation, baseline neuropathy was reported in 24% of patients who later initiated treatment with lenalidomide and 16% who initiated treatment with bortezomib. The incidence of grade 3-4 and serious AEs is reported in the Table. More lenalidomide-treated patients experienced grade 3-4 neutropenia (24% lenalidomide, 5% bortezomib, 11% other). Peripheral neuropathy (PN; all grades) was observed in 3% of patients treated with lenalidomide and 23% treated with bortezomib; 1% and 3%, respectively, were grade 3-4. VTE was low in both groups. Treatment discontinuations were 65% with lenalidomide, 81% with bortezomib, 82% with other treatments. Primary reasons for discontinuation were AEs (17% lenalidomide, 12% bortezomib, 22% other) and disease progression (16% lenalidomide, 14% bortezomib, 32% other). Incidence of death was similar with all treatments (6% lenalidomide, 8% bortezomib, 5% other). Assessments for second primary malignancies (SPM) were to be conducted up to 36 months after treatment discontinuation; no patient has developed SPM so far. **Conclusions.** In this post-authorization study, AEs observed in patients treated in daily clinical practice in Spain were similar with all treatments, with the exception that PN and neutropenia were higher in patients treated with bortezomib and lenalidomide, respectively.

Myelodysplastic syndromes - Clinical 2

0867

THE CLINICAL IMPLICATION OF SRSF2 MUTATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME AND ITS STABILITY DURING DISEASE EVOLUTION

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Background. Recurrent somatic mutation of *SRSF2*, one of the RNA splicing machinery genes, has been identified in a substantial proportion of patients with myelodysplastic syndrome (MDS). However, its clinical implication and prognostic impact remain to be clarified. **Aims** This study aimed to define the clinical correlations of *SRSF2* mutation in patients with MDS and examine the interactions of this mutation with other genetic alternations. In addition, sequential analysis of *SRSF2* mutation during the clinical follow-ups was performed to evaluate the stability of this mutation in disease progression. **Methods.** In total, 233 MDS patients, comprised 161 males and 72 females with the median age of 66 years, were included for *SRSF2* mutation analysis. Mutations of *SRSF2* and other genes were analysed by polymerase chain reaction (PCR) with direct sequencing. The results were correlated with the patients' clinical characteristics and outcomes. **Results**. *SRSF2* mutation was detected in 34 (14.6%) of the 233 patients. Twenty-five patients had missense mutations including P95H in 13 patients, P95L in nine, P95R in two, and P95A in one. Eight patients had P95_R102del(c.284_307del), a 24 base pair deletion resulting in a 8 amino acid deletion starting from P95. One patient had a novel mutation: P95_D97del (c.283_291del), a 9 base pair deletion that resulted in a 3 amino acid deletion starting from P95. *SRSF2* mutation was closely associated with male gender (19.9% in males vs. 2.8% in females, p=0.001) and older age (median, 74.5 years for *SRSF2*-mutated patients vs. 63.0 years for *SRSF2*-wild patients, p<0.001). It occurred concurrently with at least one additional mutation in 29 patients (85.3%). *SRSF2*-mutated patients had higher incidences of *RUNX1*, *IDH2*, and *ASXL1* mutations (29.4% vs. 10.1%, p=0.004; 20.6% vs. 2.5%, p<0.001; and 47.1% vs. 19.6%, <0.001, respectively). Patients with *SRSF2* mutation had an inferior overall survival (OS) (33.9 months vs. 19.0 months, p=0.010), especially in the lower risk patients defined either by IPSS (low and intermediate-1, 69.3 months vs. 32.0 months, p=0.002), by WHO classification (RA, RARS, RCMD, and RCUD, with/without RS, 93.0 months vs. 28.7 months, p=0.001), or by FAB classification (RA and RARS, 87.4 months vs. 28.7 months, p=0.001). Further exploration showed that the prognostic impact of *SRSF2* mutation might be attributed to its close association with old age. Sequential analyses of *SRSF2* mutation was performed in 173 samples from 66 patients, 37 of whom showed disease progression to higher risk subtypes during follow-ups, including 28 with transformation to AML. The analyses showed that all eight *SRSF2*-mutated patients retained their original mutations, while none of the other 58 *SRSF2*-wild patients acquired a novel mutation during disease evolution. **Conclusions**. *RSF2* mutation occurs more frequently in male and older MDS patients. There is a close association of *SRSF2* mutation with *RUNX1*, *IDH2*, and *ASXL1* mutations. The mutation is stable during the clinical follow-ups; the stability during the clinical course in MDS suggests that *SRSF2* mutation may play little role in disease progression.

0868

INVESTIGATION OF THE IMPACT OF MDS FROM THE PATIENTS' PERSPECTIVE

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Background. Little research is currently available on the impact of MDS from the patients' perspective. Where research has been conducted it has predominantly used generic cancer outcome measures. Such measures do not provide an accurate assessment of MDS patients as they miss important aspects of the condition.**Aims.** To conduct a qualitative study with MDS patients to investigate how their lives are affected by the condition. **Methods.** Qualitative, unstructured interviews were conducted with patients. Verbatim transcripts of the interviews were analysed thematically to assess the impact of MDS in terms of symptoms experienced, activity limitations and quality of life (QoL). Emergent themes were identified, clustered and harmonised. Investigation of issues related to

impairments and activity limitations were guided by the World Health Organizations (WHO) classification of outcomes related to health and injury. Investigation of QoL issues was guided by the needs-based model of QoL (Hunt SM, McKenna SP. The QLDS: A scale for the measurement of quality of life in depression. (1992). *Health Policy* 22; 307-319).This model identifies several core fundamental needs that are important to an individual's QoL and adjustment to society. Illness affects QoL by preventing the satisfaction of these needs. *Results.* The sample included 30 participants (male 17 (56.7%); mean (SD) age 65.5 (11.3) years; mean (SD) time since diagnosis 5.0 (4.5) years with low to intermediate-1 risk MDS. 30% of the sample were receiving transfusions and 33.3% were not receiving any treatment. The analyses identified several symptoms. The most frequently reported symptoms were; fatigue (97%), breathlessness (60%), increased infections (60%), sleep problems (53%), cognitive problems (50%), temperature fluctuations (47%), increased bruising (40%) and bleeding (10%), pain (30%), depression (23%) and anxiety (10%). Several common functions were affected by MDS, including; standing, walking, bending, lifting, carrying and rising from sitting. Various more complex functions were also affected such as the ability to socialise, shop and do jobs around the house. A total 33 QoL issues were identified. Quality of life issues affected six fundamental needs; physiological, safety and security, social, affection, esteem, cognitive. The QoL impact of the condition is shown in figure 1. **Conclusions.** The study was successful in developing outcome models describing the symptoms, activity limitations and QoL issues associated with MDS. Following this study a large bank of potential items has been created that will aid the development of MDS-specific outcome scales for these three different kinds of outcome.

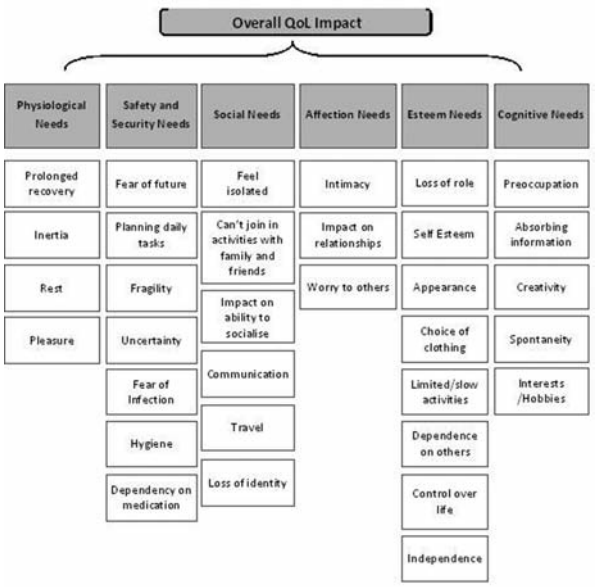

Figure 1. Needs-based QoL impact.

0869

CMML201: A PHASE 2 TRIAL OF AZACITIDINE IN CHRONIC MYELOMONOCYTIC LEUKAEMIA

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Background. Chronic myelomonocytic leukemia (CMML) has a poor prognosis and effective therapeutic strategies are lacking. Azacitidine (AZA) is licensed for non-proliferative CMML-2 (in the EU) on the basis of 11 patients randomised in the pivotal AZA-001 study *(Vidaza SmPC, Fenaux et al. Lancet Oncol 2009; 10:223-32)*. We designed a two-stage Phase 2 single-arm, multi-centre study of safety, tolerability and efficacy of AZA in CMML. **Aims and Methods.** 32 patients with CMML-1 (IPSS Int-2 / High Risk, intermediate / high Dusseldorf score, plus marrow failure / myeloproliferation or systemic symptoms) or CMML-2 (new or previously treated, PS≤2), were enrolled after informed consent was obtained. Primary endpoints were safety, tolerability and overall response rate (ORR, according to Wattel *et al,* Blood 1996) at day 28 of the 6th or last cycle of AZA (whichever came first). Secondary endpoints included incidence of complete (CR) or partial remission (PR) and haematological improvement (HI) as per the modified IWG response criteria (Cheson *et al*, Blood 2006). Responses were determined by central review. AZA was administered at a dose of 75mg/m^2 sc for 7 days (days 1-5 and 8-9) every 28 days, up to a minimum of 6 cycles. Responders and patients with stable disease were allowed to continue therapy until loss of response / progressive disease. Mutations were analysed in TET2, ASXL1, EZH2, Cbl and NRAS genes by high resolution melt and/or direct sequence analysis (n=21 evaluable patients). Bone marrow mononuclear cells from 9 patients were analysed at baseline and post course 6 for global methylation using Illumina 454K oligo arrays.

Table. Results of Phase 2 Study of Azacitidine in CMML.

Wattel Response Criteria n(%)		IWG Response Criteria n(%)	
		n=30	
CR	1 (3%)	CR	2 (7%)
GR	2 (7%)	Marrow CR	2 (7%)
MR	10 (33%)	Marrow CR, HI-P	1 (3%)
Stable Disease	2 (7%)	Stable Disease	5 (17%)
Progression	11 (37%)	Progression	9 (30%)
ORR (CR+GR +MR)	*13 (43%)*	Failure	8 (27%)
Died before last cycle (<6)	2 (7%)	HI-E, HI-P	1 (3%)
Not evaluable (<1 cycle)			2 (7%)

CR, Complete remission; GR, Good response; MR, Minor Response; ORR, Overall Response Rate; HI, Haematological Improvement

Results. 30/32 patients received AZA therapy and were suitable for analysis. Median age was 70 years (range 57 to 85); 67% were male. 13 patients (43%) had received prior treatment with hydroxycarbamide. Median number of AZA cycles was 7 (range 1-16). 2 patients (7%) received <1 cycle and were not evaluable. 14 patients (47%) received ≤ 6 cycles. Reasons for stopping were disease progression (7), toxicities, side effects, complications (2), death during treatment (2), AML transformation (1) and other (2). 16 patients (53%) continued therapy beyond 6 cycles. No AZA related deaths were observed; 1 patient (3%) had a grade 3/4 non-haematological adverse event. ORR was 43% (13 patients, see Table). 5/7 patients were able to stop hydroxycarbamide. Of 20 transfusion dependent patients at study entry (blood and / or platelets) 6 became transfusion independent (30%). Of evaluable patients, 15 had TET2 and 6 ASXL1 mutations. 10/15 patients with TET2 mutation responded as compared to 2/6 WT. 6/9 ASXL1 WT patients responded as compared to 3/6 mutated. Several patients displayed decreases in global DNA methylation to varying degrees. Cluster analysis of patients according to DNA methylation status before and after treatment did not reveal any correlations with TET2 mutation status. **Conclusions.** AZA was well tolerated. Clinically meaningful responses (including CR, GR and PR) were limited (10-17% depending on response criteria). These results do not support a Phase 3 study with this agent in CMML.(EUDRACT NUMBER: 2008-006349-23; ISRCTN: 21428905)

0870

UPDATE ON SAFETY AND LONG-TERM OUTCOMES IN LENALIDOMIDE (LEN)-TREATED PATIENTS WITH RED BLOOD CELL (RBC) TRANSFUSION-DEPENDENT LOW-/INT-1-RISK MYELODYSPLASTIC SYNDROMES (MDS) AND DEL(5Q)

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Background. In a multicenter, phase 3 study (MDS-004), LEN (5 and 10 mg)

resulted in RBC transfusion-independence for ≥ 26 weeks (43% and 56%, respectively) in patients with RBC transfusion-dependent Low-/Int-1-risk MDS and del(5q) (Fenaux, et al. Blood. 2011;118:3765-76). The most common grade 3-4 adverse events (AEs) were: neutropenia in 73.9% (with rates 46.4% and 46.4% in cycles 1 and 2, respectively) and 75.4% of patients (46.4% and 43.5%); and thrombocytopenia in 33.3% (18.8% and 18.8%) and 40.6% of patients (27.5% and 26.1%) in LEN 5 and 10 mg, respectively. Median overall survival (OS) was ≥ 35.5 and 44.5 months. For combined LEN doses, the 3-year OS rate was 56.5% and the 3-year acute myeloid leukemia (AML) progression rate 25.1%. Further evaluation of AEs of clinical interest is needed.**Aims.** To evaluate AEs of clinical interest (neutropenia, thrombocytopenia, gastrointestinal disorders, rash, pruritus and hypothyroidism) and long-term outcomes (AML progression and OS) in LEN-treated patients enrolled in MDS-004, with 1-year additional follow-up (maximum follow-up duration 70.8 months) at data cut-off in June 2011. **Methods.** Patients received either LEN 5 mg on days 1-28 or 10 mg on days 1-21 of 28-day cycles. AEs of clinical interest were evaluated using NCI-CTCAE version 3. Dose reductions were required for grade 4 neutropenia or thrombocytopenia by protocol.

Table. Grade 3-4 AEs and treatment modifications.

	Neutropenia		Thrombocytopenia		Diarrhea and abdominal pain	
Lenalidomide dose	5 mg	10 mg	5 mg	10 mg	5 mg	10 mg
Grade 3–4 events, n	154	184	70	97	3	1
Median time to onset, months (range)	1.59 (0.16–11.96)	1.64 (0.26–12.25)	1.64 (0.16–11.73)	1.51 (0.26–11.07)	5.12 (1.41–7.03)	0.30
Recovery (resolution of AE), %	97.4	98.9	97.1	93.8	66.7	100
Median time from onset to recovery, months (range)	0.33 (0.03–5.03)	0.26 (0.03–3.75)	0.26 (0.03–2.99)	0.30 (0.03–4.17)	0.85 (0.36–1.35)	0.16
Dose interruption, %	7.8	11.4	14.3	13.4	33.3	100
Dose reduction, %	17.5	17.4	10.0	22.7	33.3	0
Discontinuation, %	0.6	0.5	0	0	0	0
Bleeding (all grades), %	NA	NA	15.7	17.5	NA	NA
Infection (all grades), %	24.7	11.4	NA	NA	NA	NA
NA, not applicable						

Results. All 138 patients randomized to LEN (5 mg, n=69; 10 mg, n=69) were included in the safety population. In the double-blind phase, grade 3-4 hematologic AEs in LEN 5 and 10 mg groups were reported previously and are described above. Gastrointestinal disorders (all grades) in LEN 5 and 10 mg groups occurred in 59.4% and 65.2%, with grade 3-4 diarrhea and abdominal pain in 4.3% and 1.4% patients. Other AEs (all grades) of interest in LEN 5 and 10 mg groups included: rash, 23.2% and 13.0%; pruritus, 23.2% and 27.5%; and hypothyroidism, 0% and 1.4% patients. Per-event based analysis of grade 3-4 AEs of interest and the resulting treatment modifications are shown in the Table. No grade 5 AEs of interest were reported. G-CSF was used in events of neutropenia: 46.8% and 62.5% in LEN 5 and 10 mg groups, for a median duration of 10 (range 1-847) and 6 days (range 1-534), respectively. Median follow-up duration for AML progression in LEN 5 and 10 mg groups was 31.8 (range, 0.8-70.8) and 36.9 months (range, 0.4-69.6), with 4-year AML progression rates of 32.52% and 28.97%. Median follow-up duration for OS in LEN 5 and 10 mg groups was 35.8 (range, 1.9 -70.8) and 40.7 months (range, 0.4-69.6), with 4-year OS rates of 46.73% and 49.85%; median OS was 41.9 and 44.5 months (*P*=0.1731). **Summary and Conclusions.** In patients with Low-/Int-1-risk del(5q) MDS treated with LEN, AEs of clinical interest (neutropenia, thrombocytopenia, gastrointestinal disorders, rash, pruritus, and hypothyroidism) were manageable and rarely led to treatment discontinuation. In contrast to neutropenia and thrombocytopenia events, diarrhea and abdominal pain events were rare. AML progression and OS rates corroborate published findings.

0871

PREDICTORS OF OUTCOMES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR MYELODYSPLASTIC SYNDROMES

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Background. Allogeneic stem cell transplantation (allo-SCT) for myelodysplastic syndromes (MDS) remains the only potentially curative option. This process however, is frequently associated with significant morbidity and mortality and is typically offered to a highly selected group of patients. **Aims.** We wished to identify prognosticators predicting survival in patients with MDS following allo-SCT. **Methods.** This is a retrospective analysis of the Mayo Clinic MDS transplant database (1995-2011). The study was approved by the Institutional Review Board. Outcomes analyzed included overall survival calculated from the time of diagnosis, relapse following transplant, and non-relapse mortality (NRM). Variables included: age, Revised International Prognostic Scoring (IPSS-R), cytogenetics (according to IPSS-R categories), World Health Organization (WHO) categories, therapy related MDS, presence of monosomal karyotype, conditioning regimen, HLA disparity, incidence of acute and chronic graft versus host disease (GVHD), presenting hemoglobin, platelet count, bone marrow blasts, circulating blasts and serum ferritin levels. Multivariate analysis was performed using the Cox Regression Model. Results. From 1995 through 2011, a total of 80 consecutive patients with MDS underwent allo-SCT at the Mayo Clinic. Of these, 55 were male (68.75%) with a median age of 54 years (range, 24-68 years). Twenty one patients (26.3%) had therapy related MDS. A myeloablative conditioning was used in 47 (58.7%) patient (23 - cyclophosphamide/total body irradiation and 24- busulfan/cyclophosphamide). Reduced intensity conditioning was used in 33 (41.3%) patients (30 - fludarabine/melphalan, 1- busulfan/fludarabine and 2- pentostatin/total body irradiation). Monosomal karyotype was found in 32 (40%) patients. IPSS-R risk stratification identified 48 (60%) patients in the very poor, 13 (16.3%) poor and 19 (23.7%) in the intermediate/good risk categories. In 67 (83.7%) patients, allo-SCT utilized cells from HLA-matched donors. NRM was noted in 25 (31.3%) patients. The median survival was 29 months (range, 4-333 months) with a four-year survival of 36.3%. Twenty patients (25%) relapsed following allo-SCT (15 - with very poor IPSS-R risk category, 4 with poor, and 1 with intermediate). Acute (grade 2 to 4) and chronic graft versus host disease (limited or extensive) were noted in 44 (55%) and 45 (56.3%) patients respectively. On a univariate analysis, the presence of monosomal karyotype, poor or very poor IPSS-R categories, high risk cytogenetics (poor/ very poor per IPSS-R), and therapy related MDS were associated with an inferior survival while the presence of chronic GVHD was associated with better outcomes. On a multivariate model, good/intermediate IPSS-R risk categories and the presence of chronic GVHD were independent predictors for better survival (table 1).

Table 1. Prognostic variables affecting survival in 80 patients with MDS following allogeneic stem cell transplantation.

Variable	P-Value (univariate analysis)	P-Value (multivariate analysis)
Age	0.38	
WHO classification	0.33	
Hemoglobin at diagnosis	0.43	
Platelet count at diagnosis	0.07	
Bone marrow blasts at diagnosis	0.08	
ANC at diagnosis	0.5	
Peripheral blood blasts at diagnosis	0.28	
Conditioning regimen	0.7	
HLA disparity	0.1	
Ferritin at diagnosis	0.7	
Presence of MK	**0.007**	
Cytogenetics per IPSS-R Categories	**0.0003**	
Therapy related MDS	**0.03**	
IPSS-R	**0.02**	**0.04**
Acute GVHD	0.42	
Chronic GVHD	**<0.0001**	**0.001**

* Using Cox regression Model, MDS = myelodysplastic syndromes, WHO=world health organization, ANC=absolute neutrophile count, MK=monosomal karyotype, IPSS-R=revised international prognostic scoring system, GVHD=graft versus host disease.

Conclusions. Of all established prognostic factors in MDS; IPSS-R risk categories, high risk cytogenetics (monosomal karyotype and poor/very poor IPSS-R cytogenetic groups), therapy related MDS and chronic GVHD were found to be prognostic in patients who had undergone allo-SCT. However, only good/intermediate IPSS-R risk categories and chronic GVHD were independent predictors for better outcome.

0872

RWPSS VALIDATION IN AN INDEPENDENT COHORT OF 946 MYELODYSPLASTIC SYNDROME PATIENTS FROM THE MDS PIEDMONT REGISTRY

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Background. Transfusion dependency has been proposed as a key prognostic variable for Myelodysplastic Syndrome (MDS) patients by Malcovati et al in the WHO classification-based Prognostic Scoring System (WPSS). More recently, the use of severe anemia with a defined hemoglobin (Hb) level cut off as a surrogate for transfusion dependency has been proposed in a new version of the WPSS score (rWPSS). **Aims**. aim of our study was to investigate rWPSS reproducibility and accuracy in defining OS and risk of leukemic evolution (LE) in a cohort of MDS patients enrolled in the MDS Piedmont Registry. **Methods.** 946 patients with all available data out of 1558 patients enrolled in the Registry from 1999 to 2011 were analysed. We evaluated overall survival (OS) and time to LE according to IPSS and rWPSS risk scores. Results. Median age was 75 years (range 26 - 106). 577 out of 946 patients were male. WHO classification was as follows: 212 RCMD; 188 RAEB-1; 138 RAEB-2; 408 RA, RARS, isolated 5q deletion or RCUD. We included 63 secondary MDS in the analysis. According to IPSS score, 40% of the patients were low risk, 39% Int-1, 16% Int-2 and 5% high risk. rWPSS stratification was as follows: 269 out of 946 patients were very low (VL) risk, 258 low (L), 160 intermediate (I), 208 high (H) and 51 very high (VH).OS by Kaplan-Meyer method was analysed according to rWPSS and IPSS scores. In our cohort rWPSS can very well define five prognostic risk groups (median OS of 106 months in VL, 71 in L, 44 in I, 22 in H and 14 in VH respectively). Our data are in agreement with the work published by Malcovati et al. In 2011. Conversely IPSS, even if useful to define four prognostic groups, cannot separate very well the slopes of Int-2 and high risk curves.Considering time to LE, VL and L curves according to rWPSS show similar trends without a statistically significant difference in OS, otherwise I, H and VH risk groups show well differentiated survival curves (time to 25% LE: not reached in VL; 134 months in L; 41 months in I; 16 months in H; 6 months in VH). Conversely, the differentiation of the unfavourable Int-2 and high risk groups according to IPSS score is not so evident (time to 25 % LE: 10 months in Int-2 and 5 in high risk group patients respectively; p value >0,05). **Conclusions.** Both IPSS and rWPSS are very well reproducible prognostic scores. However, in our hands, rWPSS score (including the degree of anaemia and WHO classification) can better stratify higher risk patients in comparison to IPSS considering both OS and time to LE. Our results confirm the usefulness of the degree of anaemia and WHO evaluation in defining MDS patients prognosis.

0873

CYTOGENETIC MONITORING FROM PERIPHERAL BLOOD IN MDS PATIENTS: RESULTS FROM THE MULTICENTER GERMAN PROSPECTIVE DIAGNOSTIC CD34+FISH STUDY

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Background. Chromosomal aberrations play an important role in pathogenesis, diagnosis, prognosis and treatment allocations of patients (pts) with myelodysplastic syndromes (MDS). The gold standard of cytogenetic diagnostics in MDS are conventional chromosome banding (CCB) analyses of bone marrow (bm) metaphases. Most abnormalities can also be detected by fluorescence in situ hybridisation (FISH), but a frequent cytogenetic monitoring based on CCB analyses is not possible due to patients intolerance of repeated bm biopsies. In the present prospective multicenter German diagnostic study "Screening and genetic monitoring of patients with MDS under different treatment modalities by cytogenetic analyses of circulating CD34+cells"(ClinicalTrails.gov: NCT01355913) we followed MDS pts by sequential FISH analyses of peripheral blood samples. **Methods.** CD34+ progenitor pb cells were enriched by immunomagnetic cell sorting (MACS®) and analysed by FISH using a "Superpanel"(D7/CEP7, EGR1/D5, CEP8, CEP X/Y, D20, TP53, IGH/BCL2, TEL/AML1, RB1, MLL, 1p36/1q25, CSF1R, all Abbott® Products) at initial screening, every 12 months during follow-up and in case of suspected disease progression and a "standardpanel"(EGR1/D5, D7/CEP7, CEP8, TP53, D20, TEL/AML1, CEP X/Y, plus if necessary an informative probe from the superpanel) every 2 months in the 1st and every 3 months in the 2nd and 3rd year. If bm aspirate was available, additional CCB and FISH analysis on enriched CD34+ and non-enriched bm cells were performed. Blood counts were performed monthly and full blood counts to all FISH analyses. Cut-off values for each FISH probe were evaluated at our lab. All cytogenetic informations and bm morphology, clinical course and therapies were documented in a central database. All pts gave their written informed consent. The study was approved by all local ethic committees. **Results**. After 40 months study duration 364 pts (160 m, 204 f) with suspected or cytomorphologically proven MDS from 18 German centres of haematology were included in the study: With regard to age, gender distribution and MDS subtypes the study cohort was representative of the disease. Median follow-up at the time of analysis was 8.2 months (1-36 months).In 72.3% of pts chromosomal aberrations were detected by FISH of circulating CD34+ cells. The median number of anomalies was 2 (range 1-10). The most common aberrations in order of frequency were del(5q), del(7q)/-7, +8, del(12p), del(17p), aberrations of chromosome 21 and 11, followed by rare aberrations of chromosomes Y, 13, 18, 1, X and 14. Out of 250 pts with at least 2 sequential analyses, 37 (14.8%) showed a karyotype evolution by FISH. **Conclusions**. Our first results show that FISH analyses of enriched circulating CD34+ pb cells is a reliable method for screening and cytogenetic monitoring of MDS pts. It is an easy tool for frequent follow-up analyses. With a large FISH probe panel even rare abnormalities can be detected. Small deletions such as del(12p) and del(11q) seem to be not that rare in MDS as it is known from CCB analyses. Karyotype evolution can be observed from pb, so we expect to learn more about chromosomal instability and the course of cytogenetic progression in MDS.

0874

RESULTS OF A PHASE II TRIAL OF AZACITIDINE (AZA)+/- EPOETIN BETA (EPO) IN LOWER RISK MDS

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Background. Although anemia of IPSS low and int-1 (LR) MDS initially responds to erythroid stimulating agents (ESA) in 40-50% of patients (pts), response is generally transient. Anemia recurrence with RBC cell transfusion dependency (RBC-TD) is an indicator of poor prognosis, even in absence of progression to higher risk MDS. AZA leads to RBC transfusion independence (RBC-TI) in 30-40% of LR-MDS (Lyons, JCO, 2009), but it has not been prospectively tested in LR-MDS patients resistant to ESA. It also remains unknown if the addition of ESA to AZA would be useful in such pts. **Methods.** In this randomized phase-II trial (GFMAzaEpo-2008-1 trial, NCT01015352), the Groupe Francophone des Myélodysplasies (GFM) compared AZA 75mg/m2/d for 5 days every 28 days for 6 cycles (AZA arm) to the same treatment plus EPO 60000U/week (AZA+EPO arm). Inclusion criteria were LR-MDS resistant to at least 12 weeks of ESA, with RBC-TD ≥ 4 RBC units in the previous 8 weeks. Responders in both arms were eligible for maintenance up to 12 monthly cycles, unless progression or loss of erythroid response occurred. The primary endpoint was RBC-TI (HI-E major using IWG 2000 criteria) after 6 courses.**Results.** Between 2009 and 2010, the 98 planned pts were included: M/F 65/28; median age 71y (41-84); 5 pts were excluded (one consent withdrawal, 2 early unrelated events and 2 with exclusion criteria). In the remaining 93 pts, IPSS risk was low in 69 and int-1 in 23 pts, with no imbalance between arms. Five pts received < 4 cycles and 16 received only 4 or 5 cycles, mainly due to toxicity or progression. RBC-TI after 6 courses was observed in 16.7% (8/48) in the AZA arm and 18 % (8/45) in the AZA+EPO arm (P=1), and in 19.5% (8/41) and 26 % (8/31) respectively, in the 72 pts who received ≥ 6 cycles (P=.57). Overall best response rate (at least HI-E minor using IWG 2000 criteria) was 35 and 33% in the AZA and AZA+EPO arms, respectively (P=0.99).The 26 patients in response received further AZA maintenance courses (median; 18, range; 6-18). Response duration was 18 months in AZA+EPO arm *vs* 15,2 in AZA arm (P=0.229). Of note, only 9 pts in the AZA+EPO arm required at least one hospitalization for a SAE, compared to 22 pts in the AZA arm (P=0.015).**Conclusion:** Erythroid response to AZA, in LR MDS with demonstrated ESA resistance, appears lower than expected, with no improved outcome when combined with an ESA. The latter may however improve tolerance of AZA in LR MDS.

0875

CLINICAL PROGNOSTIC FACTORS FOR MYELODYSPLASTIC SYNDROMES PRESENTING NON-UNFAVOURABLE CYTOGENETIC CATEGORIES ACCORDING TO IPSS AND THE NEW CYTOGENETIC PROGNOSTIC SCORING SYSTEM

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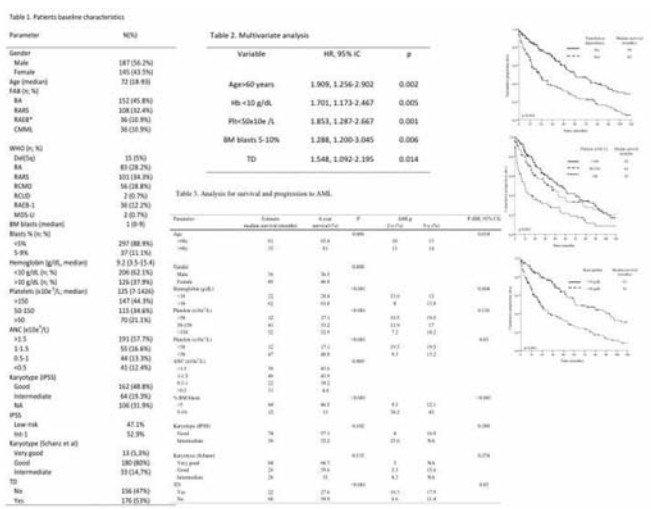
Background. Prognosis of patients with myelodysplastic syndromes (MDS) and particularly those categorized as lower risk (<10% blasts or Low/Int-1 IPSS) is very heterogeneous, as different clinical or biological factors may impact on survival[1,2]. Recently, a new cytogenetic prognostic scoring system[3] has been proposed for the upcoming revised-IPSS, which also includes the degree of cytopenias in predicting, globally, the outcome for MDS patients. However, the role of these clinical parameters is not validated for the new groups of very good, good and intermediate (except del7q) cytogenetic categories. **Aims.** To evaluate prognostic significance of clinical factors for survival and risk for progression to acute myeloid leukemia (AML) in lower risk MDS patients defined as <10% bone marrow blasts or lower risk cytogenetic categories as defined by Schanz et al[3]. **Methods.** A total of 332 MDS patients from a single institution (period: 1990-2011) were analysed for survival and risk for progression to AML. Median age was 71y (18-93). Patients baseline characteristics are shown in table 1. Demographics, peripheral blood count, transfusion dependency (TD), cytogenetic subgroups (good and intermediate by IPSS and very good, good and intermediate by Schanz) and bone marrow (BM) blasts were evaluated. Almost 90% of patients received either best supportive care with blood transfusion as clinically required and/or just observation until symptomatic cytopenia occurred. Patients treated with 5-azacitidine, lenalidomide, intensive chemotherapy or alloSCT receptors were censored at the time of initiation of such therapies. Altogether, no patients receiving treatment with potential survival advantage was included. Survival was analyzed from diagnosis until death from any cause and estimated by the Kaplan and Meier method and log rank test. Cox proportional hazards regression model was used to analyze OS as end point. Risk for AML progression was analyzed using the Gray test. The cumulative incidence was computed with the cmprsk package for R 2.6.2 software. The competing event was death without AML progression. **Results**. After 41 months mean follow up (1-238 months), 232 patients had died (69.8%). In multivariate analysis, severity of cytopenias (anemia and thrombocytopenia), age >60y, BM blasts 5-9% and TD, significantly influenced on survival (all p<0.05. Table 2). Forty-eight patients progressed to AML (14.4%). Transfusion dependency (p=0.05), platelet<50x10e^9/L (p=0.05) and BM blasts 5-10% (p<0.001) were the only factors associated with increased risk of progression to AML (Table 3). Interestingly, neither IPSS cytogenetic category (good vs intermediate) or the very good, good or intermediate categories by the new cytogenetic scoring system influenced on OS or AML progression in this set of MDS patients with <10% BM blasts. **Conclusions**. Although cytogenetics remain the main prognostic factor in MDS, the current study identifies clinical parameters predicting outcome among patients with the better cytogenetic profile. Degree of cytopenias, blasts 5-9% and TD may identify a subset of patients within the non adverse karyotype, in which aggressive approaches could possibly be required to improve survival or prevent disease progressionReferences1-Garcia-Manero et al. Leukemia 20082- Falantes et al. ASH Annual Meeting Abstracts, Nov 2011; 118: 17303-Schanz et al. J Clin Oncol 2012.



0876

THE STUDY OF CELL CHIMERISM AFTER ALLOGENEIC TRANSPLANTATION CAN IMPROVE THE MANAGEMENT OF PATIENTS WITH HEMATOPOIETIC MYELOID MALIGNANCIES.

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Background. delayed full donor cell quimerism may be associated with relapse in myeloid malignancies. The kinetics and the impact of chimerism on outcome of these patients could be relevant for adequate patients management.**Objectives**. To describe the kinetics of the cell chimerism and to analyze its impact on MDS/AML outcome patients after hematopoietic trasplant. **Patients and methos** 124 patients with MDS (n=56)/AML (n=68), allotransplanted in two different units from January 2005 to December 2012, were studied. Chimerism was performed in whole bone marrow as well as CD3+ and CD15+ selected peripheral blood cells. MRD was analysed by flow cytometry. Univariant comparisons between patients were done using T or Chi-square tests as appropriate. Variables with a significance level below 0.1 were included in a logistic regression analysis. Survival was studied using the Kaplan-Meier method. **Results.** Median age at trasplant was 55 years and median CD34+ cell dose was 5.5 x 10^6/Kg. At day + 56, complete BM chimerism was present in 70% of patients while complete CD15 PB chimerism was 89% and complete CD3 PB chimerism was only 30%. At day +100 percentage of chimerism results was as follows: 74%, 92% and 40% of complete chimerism for BM, PB CD15 and PB CD3, respectively.All patients engrafted and at day +100, all alive patients but one (n=102) were in CR. MRD monitoring at day +100 was positive in 21% of patients.After a median follow up of 2 years, overall and relapse free survival

were 57 and 60%, respectively.Regarding overall survival the presence of complete chimerism in BM at day +56 and +100 improves outcome (OS of 62% vs 51% and 64% vs 49% respectively at day +100 for patients with complete vs mixed chimerism, p=0.017 and p=0.02). When patients with mixed chimerism in BM at day +100 were analyzed for cGVHD development overall survival was improved for patients in which it was present (OS of 66% vs 28% for patients with and without cGVHD, p=0.02).Regarding relapse free survival (RFS) the presence of complete chimerism in BM at day +56 and +100 was associated with better evolution (RFS of 63% vs 60% at day +56 and 73% vs 60% at day +100 for patients with complete vs mixed chimerism, p=0.04 and p=0.004, respectively). Development of cGVHD in patients who showed mixed chimerism in BM at day +56 improved RFS in this subgroup of patients (RFS of 80% vs 33% for patients with and without cGVHD, p=0.03).Multivariate analysis for OS showed that the presence of MRD+ [HR 1.3 (0.08-0.8), p=0.02] and mixed BM chimerism at day +100 [HR 1.2 (0.09-0.9), p=0.04] were the two variables retaining statistical significanceFor RFS the same variables as well as PB CD3 mixed chimerism at day +56[HR 1.8 (0.01-1.3), p=0.08], significantly influenced relapses. **Conclusions**. Early evaluation of chimerism may help to identify patients at high risk for relapse in AML/MDS. Due to the graft anti-tumor effect the development f cGVHD could improve evolution and strategies modifying the immunosuppressive status could benefit patient´s outcome.

0877

OUTCOME OF APLASTIC ANEMIA IN ADOLESCENCE. A SURVEY OF THE SAAWP OF THE EBMT

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Background. acquired aplastic anemia has a peak incidence in adolescence and treatment algorithms are often cut below the age of 18 years without further subdivision. we analyzed the OS and EFS in 642 consecutive adolescents included in the database of the SAAWP of the EBMT from 2000 to 2009, after the following treatments: first line immunosuppression (IS), first line matched family (MFD) or alternative (ALT) donor HSCT, HSCT after failed IS. Death, relapse, MDS/AML, PNH clone, late tumours from diagnosis to death/last follow-up were considered as the events. **Aims**. to evaluate the impact of different treatments on the outcome of the disease, including the occurrence of post-therapy late tumors; to identify the best treatment strategy in this group of age. Data base anaslysis. **Results**. Three 3 year probability OS and EFS were 82% and 77%, respectively. Interval diagnosis-treatment >0.21 years negatively affected OS and EFS. OS after IS (90%) was higher than after both MFD (86%) and ALT HSCT (72%); OS for HSCT post IS was 78% (P= 0.005). In post-hoc analysis differences were significant only for IS over ALT HSCT (p=0.01) and for MFD over ALT HSCT (P=0.001). EFS was 64% after IS, 83% after MFD HSCT, 70% after ALT HSCT and 71% after HSCT post failed IS (P=0.004). In post-hoc analysis differences were significant only for MFD over both ALT HSCT (P= 0.004) and IS (P=0.003). A trend for significantly better EFS was seen for ALT HSCT over HSCT after failed IS. AcGVHD III-IV occurred in 23% of MFD, 30% of ALT HSCTs and 23% of HSCT after failed IS. Chronic GVHD occurred in 7% of MFD, 18% of ALT HSCTs and 27% of HSCT after failed IS. BM as source of cells provided a significant advantage in OS and EFS over PB and CB. OS, but not EFS, was significantly superior in adolescents transplanted in adult centres than in mixed and pediatric centres. Twelve late tumors (2%) occurred at a follow-up of 3 years in 7% of patients after IS, 5.3% after transplant in failed IS and 0.9% after front line transplants (MDF plus ALT). IS either alone or before HSCT induced a significant increased risk of late tumors (7.1, P = 0.001). In the whole cohort, the only factor negatively affecting OS and EFS in univariate and multivariate analysis was interval diagnosis-treatment > 0.21 years. IS negatively impacted on EFS in univariate and multivariate analysis. In the transplanted patients, interval diagnosis-treatment ≤ 0.21 years, BM as source of cells and MFD were favourable prognostic factor for OS and EFS in univariate analysis. Only interval diagnosis-treatment and BM cells were confirmed in multivariate analysis. **Conclusions**. aplastic anemia in adolescents has a very good outcome. If a MFD is available, HSCT performed in an adult Centre using BM cells is the first choice treatment. If there is no MFD, first option is IS that provides a survival of 90% although an inferior EFS. If IS fails, ALT HSCT is a good rescue option.

0878

THE EXPRESSION OF CD7 AND B7-H1 ON MYELOBLASTS IS INDEPENDENTLY ASSOCIATED WITH DISEASE STAGE IN AL-MDS PATIENTS: MULTICENTER VALIDATION STUDY

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Background. Immunophenotyping by flow cytometry (FCM) reveals a variety of abnormalities in hematopoietic cells in myelodysplastic syndromes (MDS). Such aberrations may help elucidate the disease pathophysiology. In our previous FCM studies, we reported that: 1) The immunophenotype of myeloblasts becomes more immature as the disease progresses, in particular when transformed into acute myeloid leukemia (AML), as exemplified by the increase in the fraction of $CD7^+$ and $CD15^-$ myeloblasts. 2) Expression of the B7-H1 protein (CD274), a B7 family molecule associated with evasion of the antitumor immune response in some neoplasia, is observed on MDS myeloblasts in some patients. Furthermore, our in vitro studies suggested that the expression of either the CD7 or B7-H1 molecule on MDS myeloblasts is associated with their increased proliferative potential. **Aims.** We conducted a multicenter FCM study to explore whether our previous immunophenotypic data from patients with MDS and AML transformed from MDS (AL-MDS) were reproducible, in particular whether CD7 and B7-H1 molecule expression on myeloblasts was associated with advanced disease. **Methods.** Bone marrow cells from 115 patients (93 MDS and 22 AL-MDS) were stained with three antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or peridin chlorophyll (PerCP). Combinations of the three antibodies were CD7/CD34/CD45 (FITC/PE/PerCP), CD10/CD34/CD45, CD15/CD34/CD45, CD34/CD11b/CD45, CD34/CD56/CD45, and CD34/B7-H1/CD45. Data were acquired using a FACSCalibur flow cytometer (Becton Dickinson, BD) and analyzed with CellQuest software (BD). Analysis of FCM parameters was performed according to previous reports (Ogata et al., Haematologica 2009, 94, 1066-1074; Della Porta et al, Haematologica, in press).**Results.** With progression of disease stage, the immunophenotypes of $CD34^+$ myeloblasts became more immature, acquiring CD7 and losing CD15 expression, and the percentages of $CD34^+$ B-progenitors among total $CD34^+$ cells and the granularity of granulocytes decreased. There was a strong negative correlation between the percentages of $CD34^+$ myeloblasts in all nucleated cells and percentages of B-progenitors in all $CD34^+$ cells in all patients. In multivariate logistic regression analysis, three parameters alone, percentages of $CD34^+$ myeloblasts in all nucleated cells, CD7 expression on $CD34^+$ myeloblasts, and B7-H1 expression on $CD34^+$ myeloblasts, were independently associated with AL-MDS patients. **Conclusions.** The present data consolidate the concept that CD7 and B7-H1 mole-

cules are associated with the disease progression mechanism in MDS. We conclude that further studies and targeting of the pathophysiology mediated by these molecules in MDS would be worthwhile.

0879

TP53 MUTATIONS ARE ASSOCIATED WITH COMPLEX CYTOGENETICS IN HIGHER-RISK MYELODYSPLASTIC SYNDROMES AND IMPACT RESPONSE TO AZACITIDINE

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Background. Myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow disorders characterized by ineffective hematopoiesis leading to peripheral cytopenias. Higher-risk MDS progress to acute myeloid leukemia within months. Treatment with azacitidine has been shown to prolong overall survival and delay progression to acute leukemia. Azacitidine also shows encouraging results in elderly patients with secondary acute myeloid leukemia (sAML) not suitable for intensive chemotherapy. TP53 mutations have been described mainly in MDS with del(5q) and shown to be associated with disease progression, poorer prognosis and poor response to therapy. **Aims.** We retrospectively analyzed the prevalence of TP53 mutations by immunostaining of bone marrow biopsies in patients with higher-risk MDS (IPSS intermediate-2, high) and sAML treated with azacitidine and correlated this to outcome. **Methods.** Patients with higher-risk MDS and sAML who received at least one complete cycle of azacitidine (75 mg/m^2 d1-7) were screened for evaluation. Paraffin-embedded bone marrow slices were stained for p53. Staining was defined as positive if more than 5% of cells showed strong nuclear staining. Sequencing of TP53 revealed mutations in 7/7 positively stained bone marrow slices so that positive staining correlated well with TP53 mutation status. **Results.** Until now 61 patients were successfully screened. Currently, immunostaining for p53 has been performed on bone marrow slices of 46 patients; it was positive in 24% (n=11) and negative in 76% (n=35). Regarding the cohort of p53 negative patients the median age was 71.5 years; 3 (9%) patients had RAEB-1, 15 (43%) RAEB-2, 1 (3%) CMML-1/-2 respectively, 15 (43%) sAML; within these patients 7 (20%) had therapy-related MDS and AML. IPSS was intermediate-2 and high in 29% (n=10) respectively, not evaluable in 43% (n=15). Complex karyotype was present in 17% with chromosome 5 abnormalities in 50%. The median number of cycles of azacitidine was 5.4 (range 1-23), responses were 3% (n=1) CR (with CCR), 6% (n=2) bone marrow CR, 46% (n=16) SD, 40% (n=40) failure, HI (excl. CR) was seen in 17% (n=6). The subgroup of p53 positive patients consisted of 2 (18%) RCMD, 4 (36%) RAEB-2, 4 (36%) sAML and 1 (9%) MDS/MPN overlap; 4 (36%) patients had therapy-related MDS and sAML. The median age was 72.6 years. IPSS was intermediate-2 in 18%, high in 36% and not evaluable in 45%. Complex karyotype was found in 91% with chromosome 5 abnormalities in 70%. The median number of cycles of azacitidine was 3.2 (range 1-6), responses were 9% (n=1) CR (with CCR), 9% (n=1) bone marrow CR, 36% (n=36) SD, 45% (n=5) failure, 27% (n=3) HI (excl. CR). **Summary and Conclusions.** Positive immunostaining of p53 as a correlate for TP53 mutations was seen in 24% of patients with higher-risk MDS and sAML. TP53 mutations were predominantly associated with complex karyotype with chromosome 5 abnormalities. Patients with TP53 mutations received fewer cycles of azacitidine due to shorter time to progression, revealing a trend towards poorer response to azacitidine. Currently, we are increasing the patient cohort (aim: 90-100 patients) to consolidate the data. An update will be presented at the meeting.

0880

SINGLE AGENT HDAC-INHIBITION WITH PANOBINOSTAT (LBH589) HAS VERY LIMITED ACTIVITY IN TRANSFUSION DEPENDENT LOW OR INT-1 MDS PATIENTS - RESULTS OF THE GEPARD TRIAL OF THE GERMAN MDS STUDY GROUP

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Background. Epigenetic mechanisms have been identified to play an important role in the pathophysiology of myelodysplastic syndromes (MDS). Recently, they have received much attention as therapeutic targets in this disease. In fact, inhibition of histone deacetylation (HDAC) with single agent valproic acid (VPA) has been demonstrated to result in substantial erythroid improvement in up to 50 % of patients with IPSS low/Int-1 disease. With the development of more potent HDAC-inhibitors this therapeutic approach might become an interesting option especially for patients with less-advanced MDS. A promising new compound is panobinostat (LBH589), a class I/II-DAC inhibitor that has shown anti-tumor activity in pre-clinical models and leukemia patients. **Aims.** The primary objective of the trial CLBH589BDE04 (GEPARD, NCT01034657) was to evaluate the erythroid hematological improvement using modified IWG criteria in patients treated for 4 months with LBH589 as a single agent. The secondary objectives included the evaluation of the objective response rate as well as the safety and tolerability profile of LBH589. **Methods.** We prospectively investigated the single agent activity of LBH589 in red blood cell (RBC) transfusion-dependent low or int-1 risk MDS patients after providing informed consent. Main inclusion criteria were RBC transfusion dependency of at least 4 units of blood within an 8 week period and being either refractory to ESA or with a low likelihood to respond to ESA. LBH589 treatment was performed orally at a starting dose of 40 mg on Monday, Wednesday and Friday every week for a period of at least 4 months. **Results.** Of 34 pts enrolled, median age was 66 years (range 51 to 81 years), IPSS categories were low (n=11) or int-1 (n=23). The majority of patients (n=20) displayed no cytogenetic abnormalities, while del(5q) was the most common abnormality and observed in 8 patients.The patient population received LBH589 treatment for a median of 26 days (range 2 - 126 days). 31 patients began treatment with 40 mg/d, only these patients will be reported hereafter because the subsequent patients started with a lower dose within the 1st amendment.18 of the 31 patients completed the 4 months' treatment period, 15 patients had dose reductions and 13 patients stopped LBH589 prematurely due to adverse events. These included infectious complications (n=2), asthenia/fatigue (n=5) or hematologic abnormalities (n=3). Therapy-induced grade 3-4 neutropenia or thrombocytopenia occurred in 7 (22.6 %) and 8 (25.8 %) pts, respectively. Of the 18 pts evaluable for response at month 4, 1 pt (5.6 %) achieved an erythroid response. No CR or PR was reported, 1 pt (5.6 %) with a del(5q) had a cytogenetic response while 12 pts (66.7 %) had stable disease. Interestingly, the patient with erythroid response had previously responded to VPA, suggesting HDAC-inhibition as the main mechanism for response. **Summary/conclusions.** We conclude that in low/int-1 MDS patients, LBH589 at a starting dose of 40 mg q3w is poorly tolerated and has very limited clinical activity. We recommend a dose of 20 mg q3w for further exploration of this compound in combination with other drugs in older patients with MDS.

0881

MYELODYSPLASTIC SYNDROMES WITH =15% RING SIDEROBLASTS: EVALUATION OF PROGNOSTIC VALUE OF HISTOLOGIC SUBCATEGORIES FOR OVERALL AND LEUKEMIA FREE SURVIVAL

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Background. Ring sideroblasts (RS) represent abnormal mitochondrial iron deposits in myelodysplastic syndromes (MDS). RS ≥15% defines refractory anemia with ring sideroblasts (RARS). In addition, other WHO-defined MDS sub-categories can also display ≥15% RS. For this study, we have referred to these as refractory cytopenias with multilineage dysplasia with RS (RCMD-RS), MDS-unclassifiable with RS (MDS-U-RS) and refractory anemia with excess blasts and RS (RAEB-1/2-RS). Amongst these categories, RARS is believed to have the best survival rate with the lowest risk of leukemic transformation, largely due to the absence of associated dysplasia in more than one lineage. **Results**. We wished to evaluate the prognostic role of WHO based histological sub-categorization in patients with MDS-RS, especially in the context of risk stratification by karyotype and the revised IPSS (IPSS-R). **Methods.** 130 consecutive patients with primary MDS and ≥15% RS were seen at the Mayo Clinic from 1997 through 2007. All patients underwent bone marrow examination and cytogenetic evaluation at diagnosis and the pathology slides, including iron stains, were centrally re-reviewed to accurately quantify bone marrow (BM) RS percentage and to confirm WHO histologic sub-categories. In addition, each patient was assigned IPSS and IPSS-R prognostic scores at diagnosis and risk stratified by karyotype according to the IPSS-R. Cox proportional regression method was used for multivariable analysis. **Results**. Of the overall 130 study patients, 87 (67%) were male and median age was 72 years (range, 21-90 years). Fifty-three (40%) patients had RARS, 54 (41%) RCMD-RS, 5 (4%) MDS-U-RS and 18 (14%) RAEB-1/2-RS. At presentation, 32 (25%) patients were red cell transfusion-dependent and 51 (39%) displayed an abnormal karyotype including 24 very poor/poor risk karyotype per IPSS-R (1 RARS, 15 RCMD-RS, 8 RAEB-1/2-RS); monosomal karyotype was noted in 16 patients (1 RARS, 8 RCMD-RS, 7 RAEB-1/2-RS). At a median follow-up of 35 months, 100 (77%) deaths and 15 (12%) leukemic transformations were documented. Median survivals were 67 months for RARS, 29 months for RCMD-RS (HR 2.0; 95% CI 1.3-3.2), 43 months for MDS-U-RS (HR 0.8; 95% CI 0.2-2.5) and 16 months for RAEB-1/2-RS (HR 4.5; 95% CI 2.4-8.4) (p<0.0001). In univariate analysis, risk factors included anemia, thrombocytopenia, increased BM blasts, red cell transfusion need, IPSS-R and cytogenetic risk categories per IPSS-R. Significance attached to hemoglobin level, platelet count, BM blasts, and specific histologic subcategory was lost during multivariable analysis that accounted for either IPSS-R or cytogenetic categories per IPSS-R, whereas red cell transfusion need retained prognostic significance. Univariate analysis disclosed significantly inferior leukemia-free survival (LFS) in patients with RAEB-1/2-RS (HR 40.0; 95% CI 4.4-364.0) and RCMD-RS (HR 12.7; 95% CI 1.6-101), compared to RARS (p<0.0001). This was confirmed by multivariable analysis that took cytogenetic risk groups into consideration. **Summary and Conclusions:** Histologic subcategories in MDS with ≥15% RS might not be prognostically relevant for overall survival, in the context of IPSS-R or cytogenetic risk stratification. However, they retained independent prognostic value in predicting leukemic transformation. The current study also showed that red cell transfusion need is an IPSS-R-independent predictor of inferior survival in MDS-RS

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0882

IMPACT OF PRE-AZACITIDINE SERUM FERRITIN ON RESPONSE AND OVERALL SURVIVAL ON PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. Prognostic factors for response and survival in patients with myelodysplastic syndromes (MDS) treated with azacitidine (AZA) remain largely unknown. RBC transfusion dependence, independently predicted shortened OS (Itzykson R et al Blood. 2011 J13;117). Transfusion dependency seems to have a major prognostic impact in patients with MDS (Malcovati L et al. J Clin Oncol 2007; 25:3503). Sanz et al in 2008 demonstrated the independent prognostic value of development of iron overload on OS and AML risk in MDS and Sorror et al in 2010 demonstrated that serum ferritin (F) concentration can indicate iron overload and is thought to predict morbidity and mortality after hematopoietic cell transplantation (HCT) but little is known about this parameter in relation to other treatments such as azacitidine.

A)

	F<500		F 500-1000		F>1000		p value
N(%)	49 (37.79%)		44 (34.11 %)		35 (3.0%)		
	N	%	N	%	N	%	
OS at 1 year	37	75.18	38	82.76	40	42.61	<0.0001
OS at 2 years	31	62.90	24	52.17	9	9.31	<0.0001
BOR	35	71.42	38	81.81	33	34.31	<0.0001
BOR at 4 m	36	73.47	40	86.96	21	22.11	<0.0001
BOR at 6 m	44	89.90	46	100	46	48.42	<0.0001
TD	32	65.31	40	86.96	76	80	0.0062

Key: BOR: Best Overall Response (mRC+ CR+ PR+ HI+ RCit) ; TD: Transfusion dependence ;F: ferritin levels; OS: Overall Survival

B)

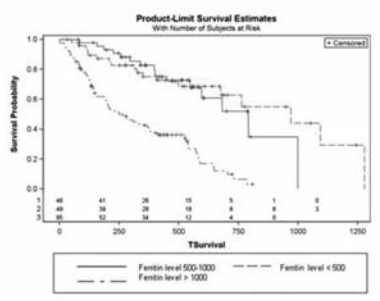

Figure 1. Overall Survival.

Aims. To assess the influence of pre-AZA ferritin levels(F) on response and survival in patients with World Health Organization (WHO) defined MDS or acute myeloid leukemia (AML) with 20-30% bone marrow blasts. **Methods.** We report a retrospective analysis of 240 patients from a Spanish compassionate use registry. **Results.** The median pre-aza serum F was 1001 ng/ml, (range: 21,

5548).The patients were divided into 3 groups according to pre-AZA levels of F (<500, 500-1000 and >1000). There was no significant difference between the low and high F groups for pre-AZA factors such as IPSS, time from diagnosis and hemoglobin levels. There was a strong correlation between a higher pre-AZA serum F (>1000 ng/ml) and a significantly inferior OS (p<0.0001). Best Overall response (BOR: mRC+ CR+ PR+ HI+ RCit) and BOR at 4 and 6 months (p<0.0001) were noted in patients with F levels < 1000. **Conclusions.** Prospective studies incorporating alternative biomarkers of iron metabolism alongside serum F levels are needed to improve our understanding of the significance of iron overload in MDS patients treated with AZA. Our results also suggest that avoiding or reducing iron overload with an appropriatechelation therapy could improve OS and response in patients with MDS treated with azacitidine. However, prospective controlled clinical trials are needed to confirm our hypothesis.

0883

STAT5 ACTIVATION IN MDS PRIMARY BONE MARROW CELLS

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Myelodysplastic syndrome (MDS) patients may respond to erythropoietin (EPO) treatment, but they often loose response or are refractory to the stimulating factor. This may be due to signal transduction abnormalities. Signal transducer and activator of trascription (STAT5) activation has been shown to be critical in hemopoiesis.We evaluated basal STAT5 phosphorylation in different marrow subpopulation and subtypes of MDS and analyzed its correlation with outcome. We have recently shown that EPO response in vivo is strongly predicted by STAT5 phosphorylation in CD71+CD45 following in vitro EPO stimulation. We analysed by a multiparameter flow-cytometry method STAT5 activation in specific cellular subpopulations of MDS bone marrow cells. Cells were stained with APC anti-human CD34, PE anti-human CD71, PerCP anti-human CD45 and Alexa-Fluor488 anti-STAT5 (pY694). Samples were analysed on a flowcytometer with 6 color laser (FACScanto). Activation of STAT5 were determined by calculating the ratio of the specific Median Fluorescence Intensity (MFI) of stimulated *versus* unstimulated cell populations. EPO response was considered positive when >1.3.We analysed in bone marrow mononuclear CD34+ cell, CD45+ and CD71+CD45- of 112 MDS cases phosphorylation of STAT5 and compared it with that of normal bone marrow cells. At the time of our study median age of patients was 72 years, 45/112 was female and the patients' diagnosis was: RA 33(29%), RARS 6(5%), RCMD 31(27%), RAEB1 17(15%), RAEB2 12(11%), 5q-syndrome 5(4%), MDS-U 1(1%), CMML 6(5%) and 1(1%) MDS/MPL. IPSS risk calculated as follow for 41 patients: Low 17(42%), INT-1 15(36%), INT-2 7(17%) and high 2(5%). The relation between STAT5 activation and overall survival was calculated for all MDS cases.Basal STAT5 phosphorylation in CD34+, CD71+ and CD45+ cells was not significantly different within WHO classification classes (p>0.05) and IPSS (p>0.05) and did not correlate with survival. EPO stimulation induced STAT5 activation in CD71+CD45- cells subpopulation of 27/90 MDS cases and in normal bone marrow cells. Non-parametric Mann Withney test showed that level of EPO-induced STAT5 activation in CD71+CD45- MDS cells was significantly lower than in normal cells (p<0.001). Evaluation of EPO response in vivo (Hb increase > 2 g/dl without transfusions after 8 weeks of EPO treatment) has shown that in 43/47 cases it correlated with in vitro EPO dependent STAT5 activation (Spearman's rho=0.401 and *p*<0.009).Although STAT5 represent an important marker of cell activity, basal STAT5 phosphorylation in CD34 + MDS cells and in other cell subpopulations did not correlate with clinical outcome and no significant clustering within WHO MDS subtypes was evident. We confirm here that evaluation of EPO response in vitro by STAT5 activation is the strongest predictor of EPO response in vivo.

0884

WHICH PATIENTS WITH SEVERE ACQUIRED APLASTIC ANEMIA DO BENEFIT FROM UNRELATED DONOR STEM CELL TRANSPLANTATION? RESULTS OF AN INTENT-TO-TREAT ANALYSIS

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Background. In the last decade, several studies have reported an improved outcome after hematopoietic stem cell transplantation (HSCT) from a voluntary unrelated donor (VUD) in patients with acquired severe aplastic anemia (SAA). This has led to a marked increase in the utilization of unrelated donor transplantation for patients with marrow failure. However, despite these recent results, the decision to transplant patients who are refractory to immunosuppressive therapy (IST) with VUD remains difficult, the alternative being repeated courses of IST that may be beneficial. In this study, we aimed to shed light on the question of the utility of unrelated HSCT by comparing the outcome of patients with or without a VUD identified. **Methods.** We conducted a multicenter donor versus no-donor comparison in 249 pediatric and adult patients with acquired SAA qualifying for a VUD search through the French and Italian donor registries over the 1994-2005 period. In median, the recipient age at diagnosis of SAA was 16 years (range=0-61) and the time interval between diagnosis and donor search initiation was 6 months (range=0-190). HLA matching was defined on low-resolution typing for HLA-A and B class I antigens and high-resolution DNA testing for HLA-DRB1. **Results**. Comparing 161 patients in whom a HLA-A, -B and -DR matched donor was identified with 88 patients without such a donor, we show no difference in survival with a median follow-up of 4.3 years from donor search initiation. However, among 179 patients who received IST using anti-thymocyte globulin (ATG), those who were 17 year-old or younger at the time of VUD search initiation had a survival benefit when belonging to the donor group (4-year probability of survival from donor search initiation = 79% ± 6% vs 53% ± 10% for the no-donor group, p=0.01). In the same ATG-treated patients (pediatric and adult), the donor group also showed a survival advantage when donor search was initiated within the more recent 2000-2005 study period (4-year survival = 74% ± 6% vs 47% ± 10%, p<0.05). **Conclusions.** HSCT from an HLA-matched VUD offers a better survival than alternative options in several subgroups of ATG-treated SAA patients. This first confirms, in an intent-to-treat manner, the conclusion of a previous prospective study conducted in children with ATG-refractory SAA. It also suggests that, in the more recent period, unrelated donor HSCT would be the best option in ATG-treated patients independently of their age.

0885

MYELODYSPLASTIC SYNDROMES WITH MONOSOMAL KARYOTYPE TREATED WITH OR WITHOUT ALLOGENEIC STEM CELL TRANSPLANTATION: THE MAYO CLINIC EXPERIENCE

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Background. Patients with myelodysplastic syndrome (MDS) and a monosomal karyotype (MK) have inferior outcomes in comparison to other karyotypic risk categories, including complex karyotypes. The median survival of this group is reported to be ≈ 7 months. In acute myeloid leukemia, MK adversely affect-

ed survival, even after allogeneic stem cell transplantation (allo-SCT). However, outcomes of patients with MDS and MK following allo-SCT have not been well studied. **Aims.** We aimed to evaluate outcomes of MDS patients with MK treated with allo-SCT and compare that to patients treated without allo-SCT at a single institution. **Methods.** A retrospective review of the Mayo Clinic MDS database was undertaken. Patients with MK were identified. MK was defined as either having two or more autosomal monosomies or one autosomal monosomy and one additional structural abnormality. The primary outcome was overall survival calculated from the time of diagnosis. Revised International Prognostic Scoring System (IPSS-R) risk categories were calculated for the group. Categorical and continuous variables were compared using chi-square and student t-tests respectively. Survival was analyzed using Kaplan-Meier plots and compared using log-rank test. The study was approved by the Institutional Review Board. **Results**. A total of 123 consecutive patients with MDS and MK were identified. Of these, 32 underwent allo-SCT and 91 were treated with supportive care or hypomethylating agents The median age at diagnosis in the two groups was 52 (range, 28-61) years and 71 (range, 40-81) years respectively. There was no statistically significant difference in the IPSS-R, frequency of therapy related MDS, or the 2008 World Health Organization (WHO) categories between the two groups (table 1). Of the 32 patients treated with allo-SCT, myeloablative regimens were used in 19 (59.4%) patients (11-busulfan/cyclophosphamide and 8 - cyclophosphamide/total body irradiation), with 19 patients received reduced intensity conditioning (11-fludarabine/melphalan and 2- pentostatin/total body irradiation). Acute (grades 2-4) and chronic graft versus host disease developed in 18 (56.3%) and 14 (43.8%) patients respectively. Non-relapse mortality occurred in 10 (31.3%) patients. Twelve (37.5%) patients relapsed with a median time to relapse of 4 months after transplantation. The median survival of patients treated with allo-SCT was 17 months compared to 4 months when allo-SCT was not utilized. The four-year overall survival was 34.4% after allo-SCT.

Conclusions. While MK in patients with MDS is associated with poor prognosis, Allo-SCT is associated with improved survival outcomes in a select group of patients with MDS and MK that are transplant eligible.

Table 1. Characteristics and outcomes of patients with MDS-MK treated with or without allogeneic stem cell transplantation

		Allo-SCT (32 patients)	No allo-SCT (91 patients)	P Value
Median Age (years)		52 (28-61)	71 (40-81)	<0.0001
WHO Classification	RCMD	9/32 (28%)	24 (26.4%)	0.8
	RAEB-1	6/32 (18.7%)	21 (23%)	
	RAEB-2	10/32 (31.3%)	17 (18.7%)	
	MDS-U	5/32 (15.7%)	26 (28.6%)	
	RARS	2/32 (6.3%)	3 (3.3%)	
Therapy related	Yes	14 (43.7%)	35 (38.5%)	0.7
	No	18 (56.3%)	56 (61.5%)	
Median hemoglobin at diagnosis (g/dL)		8.6	9.3	0.9
IPSS-R	Very Poor	25 (78.1%)	79 (86.8%)	0.2
	Poor	5 (15.6%)	2 (2.2%)	
	Intermediate	2 (6.3%)	8 (8.8%)	
	Good	0	2 (2.2%)	
Median Survival (months)		17	4	<0.0001
Four Year Overall Survival		11/32 (34.4%)	0%	

* MDS-MK=myelodysplastic syndromes with monosomal karyotype, allo-SCT=allogeneic stem cell transplantation, IPSS-R= revised international prognostic scoring system, WHO=world health organization, RCMD=refractory cytopenia with multilineage dysplasia, RAEB=refractory anemia with excess blasts, MDS-U=myelodysplastic syndromes-unclassified, RARS=refractory anemia with ringed sideroblasts

0886

INCIDENCE OF PNH CLONES BY DIAGNOSTIC CODE UTILIZING HIGH SENSITIVITY FLOW CYTOMETRY

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a chronic complement-mediated and life-threatening hematopoietic stem cell disorder characterized by deficiency of the glycocosylphosphatidylinositol (GPI)-anchored complement inhibitory proteins CD55 and CD59. Chronic hemolysis arising from this deficiency leads to serious clinical morbidities, including thromboembolism, chronic kidney disease, and increased mortality. The International Clinical Cytometry Society (ICCS) recommends multiparameter, high-sensitivity flow cytometry (HSFC) as the standard for diagnosing PNH and suggests screening for PNH in patients with bone marrow failure (BMF), unexplained cytopenias, unexplained thrombosis (arterial and venous), hemoglobinuria, Coombs' negative hemolytic anemia, and hemolysis.**Aims.** To determine the prevalence of PNH clones using multiparameter HSFC (maximum sensitivity: 0.01%) in a population of 7109 patients with unexplained cytopenias, anemia, hemolysis, and BMF by ICD-9 diagnostic category. **Methods.** HSFC was conducted using a combination of gating antibodies and GPI-linked antibodies/reagents resulting in a sensitivity of at least 0.01% in red blood cells and neutrophils. CD59 deficiency is determined in CD235a+ RBCs, FLAER/CD24 deficiency in CD15+/CD45+ neutrophils, and FLAER/CD14 deficiency in CD64+/CD45+ monocytes. Validation of this assay was based on the ICCS guidelines for PNH testing. Patients were grouped by ICD-9 diagnostic code (Table 1) and were screened for the presence of PNH clones. The change in PNH clone sizes in patients who had follow-up in studies 3-12 months after initial screening were also investigated. **Results.** A total of 7109 patients were screened for PNH clones. Of these patients, 5653 had a diagnosis aligning to one of the ICD-9 diagnostic categories identified in Table 1. Of these patients, 426 (7.5%) had PNH clones. The incidence of PNH clones categorized by ICD-9 diagnostic code are presented in Table 1.

Table 1. Incidence of PNH Clones in Patients with ICD-9 Diagnostic Code at Dahl-Chase Diagnostic Services

ICD-9 Diagnostic Code	General Description	Incidence of PNH Clone
284, 284.01, 284.8, 284.81, 284.89, 284.9	Aplastic Anemia	26.1% (94/360)
238.7, 238.72, 238.73, 238.74, 238.75, 238.76	Myelodysplastic Syndrome	5.5% (32/584)
287.5	Unexplained Cytopenia	5.3% (14/263)
284.1	Pancytopenia	6.0% (61/1011)
285.2, 285.21, 285.29, 285.9	Anemia Unspecified	3.1% (37/1176)
283, 283.1, 283.10, 283.11, 283.19, 283.9	Hemolytic Anemia[a]	22.0% (143/651)
791, 791.2	Hemoglobinuria	11.3% (7/62)
790.6, 790.99, 790.4	Hemolysis	6.5% (15/231)
325, 415.1, 415.11, 434, 434.01, 444.22, 451.11, 451.19, 452, 453, 453.0, 453.2, 453.4, 453.41, 453.89, 453.9, 557, 557.1	Thrombosis	1.7% (17/1020)
280.9	Unspecified Iron Deficiency	2.0% (6/295)

[a]*Includes patients who were known to be PNH positive.*

Anemia unspecified and thrombosis comprised the most common reasons for testing. In BMF syndromes, patients with aplastic anemia had the highest incidence of PNH-positive clones (26.1%), followed by patients with pancytopenia (6.0%), myelodysplastic syndrome (5.5%), unexplained cytopenia (5.3%), and unspecified anemia (3.1%) (Table 1). The incidence of PNH clones for other symptoms was 22.0% for hemolytic anemia, 11.3% for hemoglobinuria, and 6.5% for unspecified hemolysis. **Conclusions.** In this single-laboratory retrospective study, when analyzing patients by ICD-9 diagnostic code, PNH-positive clones were detected in 7.5% of patients (426/5653). The high prevalence of positive PNH clones supports the ICCS guideline recommendations to screen for PNH in patients with hemolytic anemia, hemoglobinuria, and bone marrow dysfunction. Thrombosis was one of the most common ICD-9 codes tested in our study, with approximately 2% of patients positive for a PNH clone. The ICCS guidelines recommend testing patients with thrombosis and elevated hemolysis or unexplained cytopenia, or prior thromboembolism in unusual sites. Combining these clinical risk factors should increase PNH prevalence in patients with thromboembolism. This study confirms the need to consistently test high-risk populations for PNH to ensure early, accurate diagnosis and intervention.

0887

COMPARISON OF MULTIPLE PROGNOSTIC MODELS IN PATIENTS WITH MYELODYSPLASTIC SYNDROME: THE IMPACT OF COMORBIDITIES

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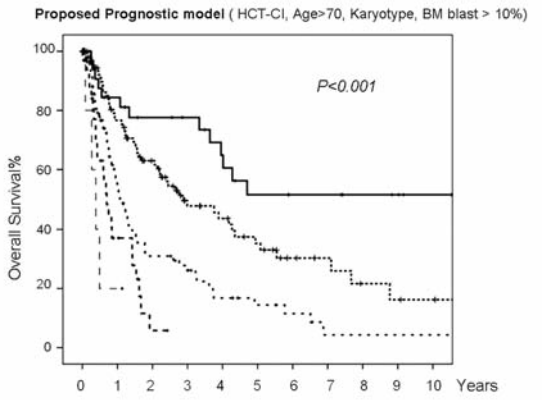
Background. Several prognostic models have been developed for patients with myelodysplastic syndrome (MDS), a heterogeneous and complex hematologic clonal disorder primarily in elderly population. Growing evidence suggests that baseline comorbidities at diagnosis influence outcome negatively.

Aims. We investigated the optimal prognostic model in these patients in a tertiary medical center. **Methods.** The study enrolled 263 consecutive patients diagnosed of myelodysplastic syndrome between 1996 and 2010. International prognostic scoring system (IPSS), WHO classification-based prognostic scoring system (WPSS), MD Anderson Cancer Center (MDACC) total MDS scores were calculated. Overall survival (OS) was compared for different risk groups in each model. The efficacy of these models was compared by the value of Akaike information criterion (AIC). Other clinical and laboratory variables associated with survival were assessed by Kaplan-Meier method, and then validated by Cox regression. A new prognostic model incorporating age, baseline comorbidities, bone marrow blast percentage and karyotype was proposed to predict survival in this cohort. **Results.** Total MDACC MDS prognostic model was more informative to categorize these patients into different risk groups than IPSS and WPSS according to the analysis of AIC value. Univariate analysis of this cohort revealed multiple prognostic factors, including age, performance status, lactate dehydrogenase (LDH), presence of blast in peripheral blood, blast percentage in bone marrow, high risk karyotype, Hematopoietic cell transplantation-comorbidity index. In multivariate analysis, more than 70 years [hazard ratio(HR), 4.02], complex karyotype or chromosome 7 anomalies [hazard ratio(HR), 6.22], bone marrow blast ≥ 10% [hazard ratio(HR), 10.47], and HCT-CI ≥ 3 [hazard ratio(HR), 5.27] were significant prognostic factors. We proposed a new prognostic scoring system using the four factors mentioned above to categorize patients into five risk groups with the median OS of not reached, 33.7 months, 12.9 months, 8.1 months, and 3.2 months, respectively. Conclusions. Adding HCT-CI in the new model incorporating other well-established factors in the prediction of outcome could better determine the prognosis in these patients and warrant further validation of this strategy in prospective large scale trials.



0888

CLINICAL EVALUATION OF ARRY-614, A DUAL P38/TIE2 INHIBITOR FOR PATIENTS WITH MYELODYSPLASTIC SYNDROMES, IDENTIFIES UNIQUE DISEASE-RELATED AND DRUG-RELATED BIOMARKERS

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Myelodysplastic syndromes (MDS) are diseases of defective hematopoiesis associated with apoptosis of hematopoietic progenitor cells. A number of signal transduction pathways have been implicated in these processes, including p38 mitogen-activated protein kinase (MAPK) and Tie2. ARRY-614 is a low-nanomolar, small-molecule dual inhibitor of p38 MAPK and Tie2 that has been studied in a Phase 1 clinical trial in patients (pts) with MDS and has demonstrated encouraging clinical activity. Forty-five pts with IPSS Low (n = 11) or Int-1 (n = 34) Risk MDS were studied. All but 2 pts had disease that was refractory/relapsed from prior therapy (median 3 prior regimens); 80% received prior hypomethylating agents (HMA). Hematological improvement (HI) was assessed by International Working Group 2006 criteria and responses were observed in all lineages (erythroid, neutrophils, platelets), including 5 bi-lineage responses (duration 8-80 weeks). All responders (n=13 of 44 evaluable pts, 30%) received at least one prior HMA. At the highest dose (1200 mg QD), 6 of 16 evaluable pts (38%) achieved HI in one or more lineage (67% bilineage).To identify possible biomarkers of disease or response, plasma from patients in the Phase 1 trial and age-defined healthy subjects was submitted for multi-analyte profiling using quantitative multiplex immunoassays. Differences in baseline levels between patients, age-matched subjects (60-75 years old) and younger subjects (≤ 60 years old) were considered significant by the Kruskal-Wallis test if $p<0.01$. Numerous analytes in patient baseline samples were different from younger healthy subjects but many changes were found to be related to age rather than disease, including eotaxin, eotaxin-3, IL-13, MMP-3 and others. This reflects the need for utilizing age-matched controls in this population. Some differences in baseline analyte concentrations were found to be related to MDS compared to age-matched subjects, including EPO, Ferritin, B2M, VCAM-1, CD40 and the Tie2 ligand, Angiopoietin-2.A subset of analytes was evaluated for change from baseline during ARRY-614 treatment. Of interest were analytes bearing AU-rich sequences in the 3'-untranslated region (e.g. IP-10, TARC, MIP1beta, IL-8 and EPO); known targets for p38-dependent regulation by mRNA stabilization and surrogate markers for p38 inhibition. Decreases in MIP1beta over 24 hours correlated with increasing ARRY-614 administered dose, suggesting this analyte to be a useful biomarker of drug exposure. The decrease in MIP1beta, while likely related to acute drug exposure, was not sustained and returned to baseline within 30 days. Other analytes had a similar pattern of regulation (e.g. IL-8, IP-10, etc.), suggesting that these may be associated with acute p38 inhibition but are not associated with improvements in disease. Importantly, some analytes remained suppressed for 90+ days (e.g. EPO, MCP-4), and thus may be associated with improvements in disease. This possible association between long-term cytokine modification and disease status or response is being further explored.In summary, ARRY-614 is a dual p38/Tie2 inhibitor, actively in development in MDS, that has demonstrated clinical activity and on-target PD effects in pts with MDS. The identification of unique biomarkers that are associated with disease improvement could greatly aid the understanding of the pathophysiology of MDS.

0889

EVALUATION OF THE EFFECTS OF SEVERE APLASTIC ANEMIA TREATMENT IN CHILDREN WITH HORSE AND RABBIT ATG. EXPERIENCE OF POLISH PEDIATRIC HEMATOLOGY GROUP

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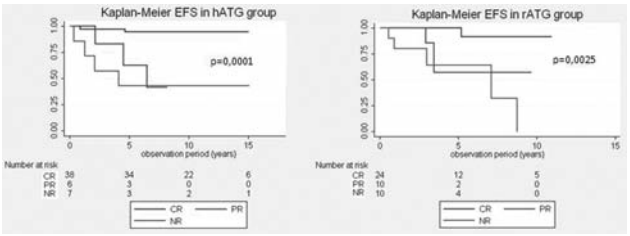
Background. Severe form of acquired aplastic anemia (SAA) in children is treated with the bone marrow transplantation from the family donor as the method of choice. The combinated therapy with antithymoglobulin (ATG) with cyclosporine is used in the absence of a matched related donor. The horse globulin (hATG) has been the first choice treatment recently, but already is almost unavailable in Europe. That is why the rabbit ATG (rATG) started to be used, which was applicable in the second course of immunosuppressive therapy (IST) with good results in cases of failure after hATG **Aims.** The aim of this study was a retrospective analysis of treatment effects in children with SAA with hATG and rATG. **Methods.** The study included 123 children with SAA in the period from 1995 to 2011, who were treated in 11 centers of pediatric hematology and oncology inPoland. (Age from 2 to 17 years). 60 of them received hATG (15mg ATG/kg/5 days and cyclosporine-CSA 5 mg / kg for 12-24 months) and 63 were given rATG (3.75 mg/kg/5 days and CSA 5mg/kg for 6-12 months). Response rate was assessed on 112, 180 and 360 day and defined as complete remission (CR), partial remission (PR), no response (NR).Results The response to the treatment (complete and partial remission in total) at the 84th day was 69% in hATG group versus 45%in rATG group ($p = 0.0070$) respectively, 69% versus 50% in the 112th day ($p = 0.03$) and 75% versus 57% in the

180th day (p = 0.04). There was no difference in the response in the 360th day-86% versus 77% (p = 0.25). The registered deaths in both groups were 10 in hATG group versus 15 in rATG group respectively. The estimated probability of overall survival did not differ between groups of patients treated with hATG and rATG (p = 0.09) for 11 years of observation period. The estimated event-free survival was 0.73 (95% CI 0.60, 0.83) for hATG and 0.57 (95% CI 0.35, 0.74) for rATG (p = 0.18) in the same period of time. In graph we perform EFS in groups treated with hATG and rATG according to response at 360th day of therapyThe estimated value of the probability of absence of the relapse (PFS) after 9 years of follow-up were 0.87 (95% CI 0.74, 0.93) in the hATG group and 0.81 (95% CI 0.44, 0.95) in the rATG group respectively. Relapses occurred in 13% patients after hATG treatment and in 19% patients treated with rATG approximately (p = 0.31).. The bleeding complications occured significantly more often after rATG in relation to hATG (p = 0.021).Summary Although this was not a randomized study, but it proved that the use of hATG as first-line treatment gives better results in children with SAA. OS in both groups was not significant, but at the border for the benefit of hATG. The bleeding complications were observed more often after rATGEtiology, age and sex have no influence on the survival.



0890

MANAGEMENT UNDER REAL CONDITIONS OF IRON OVERLOAD IN PATIENTS WITH MDS. FOLLOW UP AT SIX MONTH OF A FRENCH OBSERVATIONAL STUDY

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Background. Controlling iron overload during MDSs is of critical importance for patients dependent on frequent and/or regular transfusions. **Aims.** To describe how iron overload is managed in real conditions as part of this disease. **Methods.** An observational, prospective study was carried out in France, involving 50 physicians. MDS patients were included when they presented with an iron overload defined by, at least, serum ferritin ≥ 800 ng/ml or transfusion of > 15 PRBC. **Results**. 270 patients were included between October 2010 and January 2011. Results for 247 patients were included in the final analysis. Median age was 75 years (33-92), and 57% of patients were male. The distribution according to WHO classification was: 9.7% RA, 16.6% RCMD, 22.9% RARS, 7.4% RCMD-RS, 15.4% RAEB-1, 16.6% RAEB-2, 4.6% MDS 5q-, and 6.9% unclassifiable MDS. MDS was of low risk in 78% of patients, and high risk in 22% of patients. Patients' IPSS score was 0 for 38.8%; 0.5 for 24.9%; 1 for 14.4%; and greater than 1 for 22%. Median hemoglobin before the first transfusion was 7.8 g/dl. 73% of patients had received more than 2 PRBC/month over the last six months. 74% of patients were/are treated with ESAs, 27% with hypomethylating agents, and 13% with IMids. At inclusion, 73.5% of patients had serum ferritin levels at >1000 ng/ml; median serum ferritin was 1571 ng/ml. For low-risk MDS, median serum ferritin was 1473 ng/ml, compared to 1900 ng/ml for high-risk cases. At inclusion, 53.3% of patients were treated with iron chelation therapy; median serum ferritin for this group was 1742 ng/ml (670-9549). In the 46.6% not receiving chelation therapy, median serum ferritin was 1556 ng/ml (263-9500). Chelation therapy was initiated in the 12% of patients with a median serum ferritin of 1643 ng/ml. The main reasons for initiating this treatment were: serum ferritin level (93%), number of PRBC transfused (58%) with PRBC transfusions >20 (38%), age (25%), and, finally, IPSS score (23.5%). Hepatic and cardiac MRI were performed in 12% and 18% of patients, respectively. At six months, compliance was good, with only 2% of treatment discontinuations. In 125 patients* receiving chelation therapy at six months, a reduction in median serum ferritin from 1493 ng/ml (66-8443) to 1274 ng/ml (9-7524) was observed. A reduction of transfusion requirements was observed in 51 patients, of which 29 were under chelation therapy, and 14 were not receiving any treatment with a potential effect on hematopoiesis. **Summary and Conclusions.** Recommendations suggest starting chelation therapy when serum ferritin exceeds >1,000-1,500 ng/ml. In this study, therapy was more likely to be initiated above 1,600 ng/ml, probably due to the fact that patients were at high-risk (22%), and older (23% over 80 years).This large cohort is representative of iron overload management in MDS patients in France in real conditions, including management of high-risk MDS.

0891

THERAPY-RELATED MYELODYSPLASTIC SYNDROME: AN ANALYSIS OF 5 RISK MODELS IN PATIENTS TREATED WITH AZACITIDINE

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Background. Therapy-related myelodysplastic syndrome (t-MDS) arises as a complication of prior chemotherapy and/or radiation, and outcomes are generally poor. Several risk models for MDS have been developed but only the Global MD Anderson Risk Model and the recently reported therapy-related MDS risk model have been validated in patients with t-MDS, and none have specifically evaluated patients treated with azacitidine. **Aims.** The primary objective of the study was to compare the performance of 5 current MDS risk models (International Prognostic Scoring System (IPSS), revised International Prognostic Scoring System (rIPSS), WHO Classification-based Prognostic Scoring System (WPSS), Global MD Anderson Risk Model, and the therapy-related MDS risk model) in predicting overall survival in t-MDS patients treated with azacitidine. **Methods.** Outcomes of patients with t-MDS treated with azacitidine at the Moffitt Cancer Center were analyzed retrospectively. Each patient was assigned the appropriate score based on each risk model. The Kaplan-Meier method and log-rank test were used to evaluate survival according to each risk model and descriptive statistics were used for baseline characteristics. All statistical analyses were conducted using SPSS software, version 20.0. **Results.** We identified 84 patients (88% Caucasian, 56% male, median age 65 years) with t-MDS treated with azacitidine. Chemotherapy was previously administered in 51% of patients, radiation in 12%, and both chemotherapy and radiation in 37%. The median overall survival of the cohort was 14.7 months. When the five risk models were applied, only the Global MD Anderson risk model predicted overall survival with statistical significance. Using this model, patients with either low or int-1 risk (n=8) had a median survival of 28.3 months, compared to 17.0 months in patients with int-2 risk (n=23) and 11.5 months in patients with high risk (n=53), *p*=0.04 **(FIGURE 1)**.

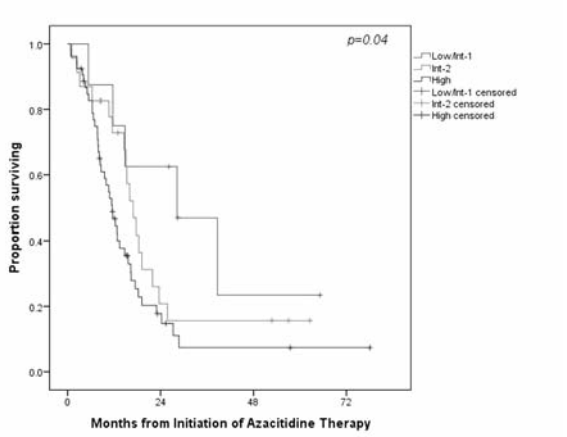

Figure 1. Survival according to Global MD Anderson Risk Model.

Using the therapy-related MDS risk model, the respective median overall survival for patients with low (n=6), intermediate (n=41), and high (n=28) risk scores were 14.8 months, 16.4 months, and 11.6 months, respectively (*p*=0.08). The IPSS, rIPSS, and WPSS did not predict overall survival with statistical sig-

nificance. With every risk model, patients in the highest risk category had a median overall survival between 11.4-11.7 months. **Summary and Conclusions.** In our cohort of patients with t-MDS treated with azaciditine, the Global MD Anderson risk model appeared to be the best predictor of overall survival, followed by the therapy-related MDS risk model, although the latter did not reach statistical significance. As expected, the IPSS, rIPSS, and WPSS were not useful in predicting survival. Our analysis is limited by the retrospective nature and the relatively small number of patients, but supports previous studies reporting poor outcomes in this patient population.

0892

P53 AND PUMA EXPRESSIONS IN BONE MARROW BIOPSIES OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS)

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Background. p53 is a tumor suppressor gene and a key regulator of apoptosis. P53 mutations confer a poor prognosis in several hematologic malignancies. PUMA (p53 upregulated modulator of apoptosis) is also extremely effective in inducing apoptosis. PUMA is a critical mediator of p53-dependent and independent apoptosis. **Aims.** The objectives of this study were i) To compare the expressions of p53 and PUMA in bone marrow hematopoetic cells of MDS patients with those of healthy subjects ii) to evaluate the prognostic effect of these apoptosis regulators (p53 and PUMA) on overall and event free survival of MDS patients, iii) to evaluate the relationship between p53 and PUMA. **Methods.** The expression of p53 and PUMA was evaluated using immunohistochemistry The procedure was carried out using specific antibodies against p53 and PUMA. Bone marrow biopsies of MDS patients at the time of diagnosis (n=103) as well as at the time of transformation (n= 20) were included in the study group. Bone marrow biopsies from individuals who had no hematologic disorder (n=12) were included as control. After staining a total of 500 bone marrow precursors were counted to calculate the percentage of positively staining cells. **Results.** The percentage of cells showing p53 and PUMA expressions were significantly higher in bone marrows of MDS patients as compared to normal bone marrow samples (p= 0,012, p=0,000, respectively). In MDS samples, there was a moderate positive correlation between p53 and PUMA expressions (R= 0.478). Although there was a mild correlation between PUMA expression and high scores in IPSS, WPSS and MPSS clinical scoring systems (R= 0.326, 0,236, 0,208 respectively), such association was not identified for p53. Additionally, there was no association between p53 or PUMA expression levels and bone marrow cellularity, cytogenetic risk assessment, MDS subgroups, overall survival and event free survival. There was however, a significant increase in PUMA expression in cases which showed transformation (p=0,038) as compared to the initial diagnostic bone marrows. No such association was observed for p53 expression (p=0,601). **Summary.** Our results showed that p53 and PUMA expressions are significantly higher in bone marrow cells of MDS patients compared to healthy controls. This finding may be especially helpful in the diagnosis of hypocellular and normocellular MDS cases. The observation that PUMA expression increases during transformation while the expression of p53 is not significantly altered suggests that PUMA alterations might be a late event during the development of MDS. However, studies with higher number of cases comparing bone marrow samples from before and after transformation are needed for a more conclusive statement. **Acknowledgments.** Supported by a grant from Hacettepe University Research Foundation

0893

RUNX1 MUTATIONS IN LEUKEMIAS DEVELOPED IN PATIENTS WITH CONGENITAL NEUTROPENIAS

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Background. Most bone marrow failure syndromes (bmfs) are associated with a marked propensity to transform into myelodysplastic syndrome (MDS) or acute leukemia, with a cumulative rate of transformation that may exceed 20% (e.g., in the case of severe congenital neutropenia). **Aims**. The genetic (and epigenetic) changes that contribute to malignant transformation in bmfs patients are largely unknown and have to be resolved. **Methods.** To elucidate the underlying molecular mechanisms of cancer susceptibility and progression in secondary MDS or acute leukemia in bmfs patients we conducted a comprehensive genome-wide characterization of genetic aberrations in the malignant cells at high-resolution level. We used high density DNA microarray (Agilent 400k/180 k) and direct sequencing of putative cancer genes to analyze a series of 31 bmfs patients at different time points during the progression into MDS and AML (51 samples in total). **Results**. Large genomic alterations, namely monosomy 7/-7q, +21q or +3q were associated with leukemic progression. Beside common copy number variants like *UGT2B*, *GSTT1*, *HEATR4*, no microdeletions or microduplications were detected in the primary or secondary diseases. However, we found recurrent somatic missense and frameshift mutations in the transcription factor *RUNX1/AML1* in 12% of all bmfs patients. Of the 31 bmfs patients, 26 were diagnosed as congenital neutropenia, one as cyclic neutropenia, two with Fanconi-anemia (FA), one with Shwachman-Diamnond syndrome (SDS), one with MDS. Eight of the 26 patients with congenital neutropenia developed secondary AML, four of the 8 (50 %) revealed somatic mutations within the *RUNX1* gene. The mutations were missense mutations of the conserved Arginine residues R139G, R174L or truncating frameshift mutations. None of the FA, SDS, or MDS patients revealed *RUNX1* mutations.**Summary**. Notably, *RUNX1/AML1* mutations have recently been described in Fanconi anemia during leukemic progression. *RUNX1/AML1* may have a more general role in the malignant transformation of patients with bone marrow failure syndromes, especially in patients suffering from congenital neutropenia.

0894

A RANDOMIZED STUDY OF DECITABINE ALTERNATING WITH CLOFARABINE VERSUS DECITABINE ALONE IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROME (MDS)

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Background. Patients (pts) with higher-risk MDS continue to have a poor prognosis even in the era of hypomethylating agents such as decitabine (DAC) or azacitidine (AZA). Clofarabine (CLO) is a second generation deoxyadenosine nucleoside analog with activity in pts with MDS as previously reported by us and other groups. **Aims.** In this study we aim to compare the efficacy and safety of using DAC alternating with CLO compared with a standard approach of DAC alone. **Methods.** Eligible pts were older than 18 years (yrs) with a diagnosis of higher-risk MDS (intermediate-2 and high risk groups based on the International Prognostic Scoring System [IPSS] and/or ≥ 10% marrow blasts). Prior intensive chemotherapy was not permitted but patients could have been exposed to biological or targeted therapies (not more than 1 cycle of DAC or AZA). Patients were randomized to DAC 20 mg/m^2 IV daily x 5 for up to a total of 24 cycles or DAC 20 mg/m^2 IV daily x 5 for 3 cycles each alternating with CLO 10 mg/m^2 IV daily x 5 for 3 cycles each for a total of 24 cycles. Concomitant use of hematopoietic growth factors was permitted as per the treating physicians' discretion. **Results.** Twenty pts were enrolled of whom 15 are evaluable. Among the 5 inevaluable pts 2 were too early and 3 did not start treatment because of insurance denial. None of the pts received prior therapy for the MDS. The median age was 66 yrs (50-85). Based on IPSS, 9 pts were intermediate-2 and 6 high-risk. Based on WHO 5 pts had RAEB-2, 3 RAEB-1, 3 therapy-related MDS (marrow blasts 5%, 6%, 12%), 2 CMML-2, and 1 MDS-unclassifiable. One pt had 20% marrow blasts consistent with RAEB-T. Six pts were randomized to DAC alternating with CLO and 9 to receive DAC only. Overall, 11 pts (80%) responded (7 CR [47%], 2 PR, 2 hematologic improvement [HI]). Responses were comparable in both arms (DAC alternating with CLO: ORR 67%, CR 50%; DAC single agent: ORR 78%, CR 44%). Four pts (2 in each arm) proceeded to an allogeneic stem cell transplant (2 in CR, 1 PR, 1 stable disease). Three patients were taken off study because of progression to AML (2 pts on DAC single agent). The median number of cycles administered was 6 (2-15). Treatment was tolerated well in both arms. Most toxicities were < grade 2. Only one grade 3 toxicity (infection) occurred (DAC single agent). **Summary and Conclusions.** Both treatment arms are equally effective and well tolerated. The substitution of DAC with CLO in half of the cycles has not resulted in higher toxicities. The study is ongoing. With ongoing accrual, updated results and time-to-event parameters will be presented.

0895

KINASE INHIBITORS REDUCE TNF-ALPHA OVER-PRODUCTION IN MONOCYTES AND STIMULATE IN VITRO HEMATOPOIETIC PROGENITOR AND STEM-CELL COLONY GROWTH IN FANCONI ANEMIA GROUP A PATIENTS

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Fanconi Anemia (FA) is a chromosomal instability syndrome with hypersensitivity to alkylating agents. Many laboratories have demonstrated involvement of FA proteins in DNA-repair mechanisms. Recent work has also demonstrated other functions of some FA proteins suggesting alternative roles in other regulatory pathways, particularly those influencing hematopoiesis. Eighty percent of FA patients develop bone marrow failure with high incidence of evolution in myelodysplasia and/or acute leukemia. These abnormalities have been related to TNF-alpha hypersensitivity in the stem and progenitor-cell pools and to toll-like receptor (TLR)-dependent overproduction of TNF-alpha by FA macrophages. The TNF-hypersensitive phenotype involves two kinases, PKR and ASK1, hyperactivated in FA cells inducing apoptotic responses both in ground state and after TNF-alpha stimulation. Recent evidence showed that TNF-alpha gene expression in *Fancc*-deficient mononuclear phagocytes induced by TLR8-ligand R848 and TLR4-ligand LPS, is inhibited by kinase-inhibitors dasatinib and BIRB796. We sought to evaluate the activity of these agents in primary monocytes and colony assays from children with FA-A. **Aims.** (i) To determine whether primary monocytes from FANCA-deficient patients: (a) exhibit TNF-overproduction phenotype in response to LPS and R848, and (b) respond to dasatinib and BIRB796 by suppressing TNF-production. (ii) Evaluate the effect of dasatinib and BIRB796 on colony growth of marrow mononuclear cells (MNCs) and CD34+ cells from FANCA-deficient patients. **Methods.** Eight FA patients with mild to severe marrow failure were included. Healthy subjects were recruited as normal controls that were run in parallel in each case. CD14+ monocytes freshly isolated from peripheral blood were cultured for 24 hours with LPS and R848 with or without dasatinib and BIRB796. Supernatant media were collected and frozen at -80 degrees. After thawing samples, TNF-alpha content was quantified by ELISA. Marrow mononuclear and CD34+ cells, freshly isolated by magnetic beads from marrow MNCs, were cultured in semi-solid culture medium supplemented with grow factors and with BIRB, dasatinib and anti-TNFalpha. Colonies were counted after 14 days.Results. Baseline TNF-alpha concentration (without TLR-stimulation) was higher in FA-A patients' monocytes than controls. After LPS and R848 stimulation FA-A monocytes produced substantially more TNF-alpha than control samples. Both dasatinib and BIRB796 suppressed spontaneous and TLR-induced (both LPS and R848) TNF-alpha production in FA monocytes. In MNC colony assay BIRB alone and BIRB in association with antiTNF-alpha increased erythroid and myeloid colony numbers and in CD34+ colony assay increased erythroid colony growth. Dasatinib alone and in combination with antiTNF-alpha consistently suppressed colony growth.Conclusions: These findings demonstrate that in primary FANCA-deficient monocytes TLR-induced TNF-alpha gene expression is aberrantly regulated and TNF-alpha overproduction can be controlled by therapeutically achievable doses of BIRB796 and dasatinib. Colony growth from MNCs and CD34+ is increased by BIRB but suppressed by dasatinib. Thus, given that in FA patients TNF-alpha hypersensitive stem-cells are over-exposed to TNF-alpha, particularly during inflammatory events, and that exposure to TNF-alpha was shown not only to suppress hematopoiesis in FA but also to favour the emergence of neoplastic clones, these results point to BIRB796 as a potential candidate for preclinical trials seeking to enhance hematopoiesis and suppress clonal evolution.

0896

PLATELET RESPONSE AT SECOND CYCLE OF DECITABINE CAN PREDICT RESPONSE AND SURVIVAL FOR MYELODYSPLASTIC SYNDROMES (MDS) PATIENTS

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Background. Decitabine treatment is effective in patients with MDS. However, there is wide range of response rate of decitabine as 30-54%. However there was no predictive marker for overall response rate and overall survival. **Aims.** We aimed to describe the efficacy and safety of decitabine and to investigate predictors for response and survival. **Methods.** We analyzed clinical data including treatment outcome in MDS patients who received 5-day regimen of decitabine in Samsung Medical Center between August 2008 and August 2011 retrospectively. **Results.** We analyzed 100 MDS patients (total 708 cycles). Patients received decitabine for a median of six cycles (range 1-25). The overall response rate (complete/partial/hematologic improvement) was 58%. Median time to any response was 1 (range 1-6). With a median follow-up duration of 380 days (range 110-1320), median overall survival was 17.7 months. Patients who showed hematologic improvements (HI) had significantly longer survival than those who did not (16.7 vs 22.5 months, P=0.002). The difference in OS was evident in the Intermediate-2/High risk group but not in the Intermediate-1 risk group. The OS in patients with marrow complete remission (m-CR) was not different to those without m-CR. Among 708 treatment courses, there were 128 fever episodes requiring intravenous antimicrobials. Multivariate analysis confirmed platelet response at second cycle [Hazard Ratio (HR) 0.339, 95% confidence interval (CI) 0.125-0.917; P=0.033] as a independent predictor for response and OS. 91.4% of patients that demonstrated improvement (CR/m-CR/PR/HI) by cycle 2. **Conclusions.** In conclusion, response of decitabine is fast and effective, and the OS was significantly longer in patients showing HI.

0897

EFFICACY AND SAFETY OF ECULIZUMAB IN CHILDREN AND ADOLESCENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a progressive, life-threatening disease characterized by chronic intravascular hemolysis caused by uncontrolled complement activation. The cellular abnormality in PNH originates from a somatic mutation in the *PIG-A* gene that results in a deficiency of the glycosylphosphatidylinositol (GPI)-anchored complement regulatory proteins CD55 and CD59. Clinical manifestations of PNH include hemolysis, cytopenia, thromboembolism, multiorgan damage, bone marrow failure, and death. Patients with PNH also experience a range of debilitating symptoms, including fatigue, shortness of breath, and abdominal pain, that significantly reduce quality of life. Systematic research in pediatric PNH patients has been limited, largely due to small patient numbers. However, pediatric PNH patients experience many of the same clinical features and life-threatening complications as adult patients. **Aims.** Assess the safety, pharmacokinetics, and efficacy of eculizumab treatment in children and adolescents with PNH. **Methods.** This 12-week, open-label, multicenter Phase I/II study included children and adolescents (aged 2-17 years) with a diagnosis of PNH. Inclusion criteria: ≥5% GPI-deficient red blood cells or granulocytes and either serum lactate dehydrogenase (LDH) levels greater than the upper limit of normal (ULN; 275 U/L) or to have received ≥1 transfusion during the previous 2 years for anemia or anemia-related symptoms. Eculizumab was administered using weight-based dosing (300-900 mg IV) at protocol-specified regular 7-14-day intervals throughout the treatment period.**Results.** Seven pediatric patients with PNH (aged 11-17 years; 4 females) participated in this study. At baseline, patients had elevated LDH, thrombocytopenia, and anemia. Peak and trough eculizumab plasma levels increased gradually and appeared to reach a plateau between 3 and 4 weeks. Mean (standard deviation) trough plasma eculizumab concentration at week 12 was 214.5 µg/mL (68.3 µg/mL), with a range of 124.2-321.1 µg/mL. Eculizumab treatment led to a rapid and sustained reduction in LDH levels from a mean of 1020 U/L at baseline to 257 U/L already at 2 weeks. When compared with baseline values, LDH decreased by 90% in all patients, indicating full inhibition of hemolytic activity. None of the patients experienced breakthrough hemolysis. Eculizumab was well-tolerated; the most common adverse

events, reported by 2 or more patients, included headache, upper abdominal pain, cough, and pyrexia, and were all mild to moderate in severity. The pharmacodynamic and safety profile of eculizumab was consistent with that previously reported in adult patients with PNH. **Conclusions.** Consistent with results in adults, eculizumab was well tolerated in pediatric patients and successfully inhibited uncontrolled complement activation and intravascular hemolysis. These results highlight the potential of eculizumab for the treatment of children and adolescents with PNH.

0898

RETROSPECTIVE ANALYSIS ON THE IMPACT OF IRON CHELATION THERAPY ON SURVIVAL AND LEUKEMIA PROGRESSION IN TRANSFUSION DEPENDENT MDS PATIENTS IN BELGIUM

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Background. Transfusion-dependent (TD) MDS patients have a significantly shortened survival as compared to patients who are not dependent on transfusions. This effect might be propagated or exacerbated by cumulative systemic iron deposition following repeated RBC transfusions. While appropriate iron chelation can prolong survival in patients with thalassemia major, this is still a highly debated topic in MDS. **Aims**. To investigate the effect of iron chelation treatment (ICT) on overall survival and leukemia-free survival and to collect information on treatment modalities, transfusion needs and chelation practices in transfusion dependent MDS patients in Belgium. **Methods.** A Belgian cross-sectional analysis, performed in Oct-Dec 2008, identified a cohort of 193 TD MDS patients (Delforge *et al.*, Ann Hemat, 2011). Two years later, this non interventional, retrospective study allowed us to collect and analyze follow-up data from 186 patients of the original cohort, providing longer term information on patterns of disease management in TD MDS patients, including ICT and its potential impact on survival. **Results**. Of 186 patients included in this study, 38% were still alive and 4% were lost to follow-up 2 years after the first cross-sectional study. At the time of diagnosis, 127 patients (68%) were classified as low-intermediate1 IPSS score, 17 (9%) with intermediate2-high IPSS score, while for 42 patients no IPSS score was available. At diagnosis mean serum ferritin was 524 µg/L. Iron chelation therapy was started on average 3.6 yrs after diagnosis and 1.4 years after the first RBC transfusion. At initiation of ICT, the mean serum ferritin was 2302 ± 2607 µg/L. 74 patients (40%) never received any chelation therapy. For 18% of patients progression to AML was reported after a median of 24 months.MDS patients with low-intermediate1 IPSS scores at diagnosis had a median survival of 87 months. Patients from this group who received ICT for at least 6 months had a significantly longer median survival than non-chelated patients (126 *vs.* 37 months; *p<0.001*). This survival difference remained significant when looking only at low IPSS patients (171 *vs.* 37 months; *p<0.001*) or only at intermediate1 patients (126 *vs.* 37 months; *p=0.002*). AML-free survival was similarly different between the two groups. In Cox Proportional Hazard models the use of iron chelation therapy appeared to be the most prominent factor impacting survival, followed by calculated "transfusion intensity". **Conclusions**. Although we cannot exclude a patient selection bias, this study confirms in an independent way the previous findings of the Groupe Francophone des Myelodysplasies (Rose *et al.*, Leuk Res, 2010) and of the Dusseldorf registry (Fox *et al.*, Blood abstr 1747, 2010): patients who received iron chelation treatment have a better outcome. Prospective randomized trials remain necessary to confirm the benefit of ICT and to identify the reason for the survival advantage.

Table 1. Patient characteristics and results.

	Non-chelated (47)	Chelated ≥ 6 months (62)	*p-value*
Mean Age	77 ± 9	77 ± 9	*0.57*
RBC last 4 mths	11 ± 8	14 ± 9	*0.044*
Total RBC units	70 ± 90	144 ± 92	*< 0.001*
Transfusion intensity	2.6 ± 3.5	2.7 ± 1.2	*0.849*
Latest SF value	3393 ± 4601	3114 ± 2692	*0.730*
IPSS Low Int1	18 (38%) 29 (62%)	28 (45%) 34 (55%)	*0.558*
Cause of death			
MDS progression	7 (15%)	9 (15%)	
Cardiac	6 (13%)	2 (3%)	*NC*
Hepatic	0	0	
Other	21 (45%)	12 (19%)	
Median survival	37 months	126 months	*< 0.001*
Leukemia free survival	37 months	171 months	*< 0.001*
AML progression	7 (15%)	7 (11%)	*0.579*
Patients died	33 (70%)	20 (32%)	*< 0.001*

Myeloproliferative neoplasms - Biology: signalling and transcription

0899

MECHANISMS DRIVING THROMBOPOIETIN RECEPTOR DOWN MODULATION IN JAK2 V617F-POSITIVE MYELOPROLIFERATIVE NEOPLASMS

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Beyond the role of JAK2 in the induction of signaling pathways, JAK2 has been shown to play a crucial role in the receptor traffic by increasing the cell surface expression and by stabilizing the mature Endoglycosidase-H resistant form of TpoR. The constitutively active JAK2 V617F mutant is the major determinant of human myeloproliferative neoplasms (MPNs). One hallmark of MPNs consists in the post-translational down-regulation and impaired maturation of TpoR (c-MPL) in megakaryocytes and platelets of patients.We are showing that co-expression of JAK2 V617F and TpoR in hematopoietic cell lines or heterozygous knock-in of JAK2 V617F in mice leads to down-modulation of TpoR levels. The mechanisms behind this effect is represented by enhanced TpoR ubiquitinylation at cytosolic lysine residues, proteasomal degradation, along with reduced recycling and maturation.TpoR down-regulation required the presence of the two lysine residues, K40 (544) and K60 (564), in the intracellular domain as well as Y626 residue necessary for MAP-kinase and STAT3 signaling pathways. Restoration of TpoR levels by inhibitors could be detected in platelets from JAK2 inhibitor treated myelofibrosis patients that express the JAK2 V617F mutant, and in platelets from JAK2 V617F knock-in mice that were treated with JAK2 or proteasome inhibitors.In addition, we show that Tpo can induce both proliferative and antiproliferative effects via Y626 of TpoR at low and high JAK2 activation levels, respectively, or upon expression of JAK2 V617F suggesting that selection against TpoR antiproliferative signaling occurs by Tpo down-modulation. Furthermore, a microarray analysis performed on Ba/F3 cells expressing TpoR and JAK2 wild-type stimulated with high Tpo concentration shows down-regulation of 70 cell cycle related genes, which coincides with the antiproliferative role of Tpo in cells that express high JAK2 levels.Finally, we have explored the ubiquitome and the profiles of ISGylated and SUMOylated proteins upon Tpo activation based on the Ubiscan® Ubiquitinylation proteomics method to detect with a specific monoclonal antibody a diglycine (KGG) tag that is the remnant after trypsin digestion of ubiquitin/ISG15/SUMO on protein substrates. We were able to identify 145 proteins that were modified in a Tpo-dependent manner either by enhancing (88) or diminishing (57) proteins with such post-translational modifications. The identity of the proteins informs about the signaling events induced by Tpo, and again, proliferative and antiproliferative signaling appear to associate with different profiles of ubiquitinylated/ISGylated and SUMOylated proteins.

0900

LNK PH DOMAIN POINT MUTATIONS WHICH OCCUR IN MPN PATIENTS ARE PARTIAL LOSS OF FUNCTION MUTATIONS

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Background. Somatic point mutations in the PH domain of Lnk, an adaptor protein that is highly expressed in hematopoietic cells, were recently described in patients with myeloproliferative neoplasms (MPN). Lnk inhibits signaling through the thrombopoietin, as well as the erythropoietin receptors (cMPL and EPOR, respectively). It directly binds to the WT JAK2 and the activated JAK2 V617F mutant, decreasing their autophosphorylation and downstream signaling through STAT5, ERK and the PI3K/AKT pathways. Knockout (KO) of Lnk cooperates with JAK2 activating variants in the formation of myeloproliferative disease in a murine bone marrow transplantation model. This further supports the importance of Lnk in the inhibition of the JAK2 STAT pathway (Bersenev A et al. J Clin Invest;120(6):2058-2069). Most of Lnk activity is thought to occur through direct binding to phosphorylated tyrosine residues on target proteins via Lnk SH domain, and a synthetic point mutation in Lnk SH2 domain (R392E) greatly abolishes Lnk inhibitory activity. Interestingly, most of the Lnk point mutations found in MPN patients occur in the Lnk PH and not the SH2 domain. In order to delineate the mechanism by which the Lnk PH domain mutations contribute to the pathogenesis of MPN, we studied the effect of these mutations on JAK2 signaling pathways in cells with WT JAK2 (BaF3 with stable EPOR expression [BaF3-E] and murine BM), as well as in JAK2V617F harboring (HEL) cells. **Results.** Overexpression of WT Lnk inhibited cell growth in WT and JAK2 mutated cells. In contrast, the PH domain mutants E208Q and G220V partially lost the ability to inhibit cell growth. In order to study if the PH domain mutants have a dominant negative effect on WT Lnk function, we reintroduced WT Lnk and Lnk mutants into Lnk KO and WT murine BM cells and assessed their colony formation potential. We found that WT Lnk inhibited colony formation to a greater extent in WT compared to KO Lnk cells. The number of BM colonies in all the Lnk mutants was either equal to or less than colony numbers in the vector control, thereby not supporting a dominant negative effect of the Lnk mutants on WT Lnk. We also examined the effect of Lnk mutants on downstream signaling of JAK2. Overexpression of WT Lnk resulted in decreased STAT5 phosphorylation in BaF3-E stimulated with Epo, in murine BM cells stimulated with IL-3 and in serum starved, unstimulated HEL cells compared to a vector control. Lnk mutants inhibited STAT5 phosphorylation less than WT Lnk in all the cells studied, further supporting the loss of Lnk inhibitory effect conferred by mutations in the PH domain. Lnk mutants retained binding capacity for JAK2, an established Lnk target, as well as for the adaptor proteins 14-3-3 and c-Cbl. **Conclusions:** Our data suggest that the loss of Lnk inhibitory function conferred by the PH domain mutations may collaborate with JAK2V617F and c-Cbl mutations in order to promote the development or progression of MPN. Studying Lnk status in MPN patients may have prognostic and therapeutic implication.

0901

THE 46/1 HAPLOTYPE IS INVOLVED IN TRANSCRIPTIONAL REGULATION OF JANUS KINASE 2 GENE

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Background. The role of *JAK2* gene and its genetic variant, *JAKV617F* mutation, in biology of myeloproliferative neoplasms (MPN) is well studied. It was shown that *JAK2* gene acts both as somatic as well as germline susceptibility locus for this specific type of diseases. Association of the *JAK2* SNP rs12343867 T>C, a part of 46/1 haplotype, with a predisposition to develop MPN and to acquire *JAKV617F* mutation, has been demonstrated. How this specific haplotype influences susceptibility to develop MPN is not clarified yet, but regulation of *JAK2* gene expression could be one of possible mechanisms. The expression of the *JAK2* gene is elevated in MPN patients regardless the presence of the V617F mutation, and this elevated level of the mRNA is one of the potential mechanisms of action of 46/1 haplotype. **Aims.** In this study, we genotyped 46/1 haplotype in our cohort of 35 MPN patients and 14 healthy controls in order to determine the C/T allelic frequency. Also, we functionally analyzed polymorphic site T>C within the intron 14 of the *JAK2* gene in order to shed light on its transcriptional activity. Our goal was to discover weather this intronic region possesses transcriptional regulation capability that could contribute to the pathogenesis of MPN and explain some specific issues, such as predisposition to develop MPN in families, or the presence of disease in the absence of *JAK2* and other oncogenic mutations. **Methods.** Screening of the JAK2 46/1 haplotype was performed using direct sequencing. PCR products containing T or C allele at the polymorphic site were subcloned into pBLCAT5 reporter vector. Functional analysis was performed in K562 cells and enhancer activity was compared to pBLCAT5 vector activity. For electrophoretic mobility shift assays (EMSA), nuclear extracts from K562 cells and radiolabelled probes corresponding to the T or C allele were used. In addition, "supershift"assay was performed using MEIS1/2 antibody. **Results.** In patients, the rs12343867 CC genotype frequency was significantly higher than that observed in controls (37% vs. 7%). Functional analyses of *JAK2* intron 14 polymorphism showed that it acts as a transcriptional repressor *in vitro*. A decrease of repressor activity was detected for C allelic variant compared to T allelic variant, potentially leading to an increase of *JAK2* mRNA level. EMSA experiments showed an involvement of activating transcriptional factor Meis1 in this process. **Conclusions:** This is the first evidence of a transcriptional regulatory element within an intron of *JAK2* gene. Our results implies the role of the SNP

rs12343867 T>C in the regulation of *JAK2* gene expression and points out that transcriptional regulation of *JAK2* gene should be taken into consideration regarding pathogenesis of MPN and heterogeneity of clinical presentation of MPN.

0902

ONCOSTATIN M (OSM) IS A FIP1L1/PDGFRA-DEPENDENT CYTOKINE AND MODULATOR OF THE MICROENVIRONMENT IN CHRONIC EOSINOPHILIC LEUKEMIA

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Background. Chronic eosinophilic leukemia (CEL) is a myeloproliferative neoplasm characterized by expansion of neoplastic eosinophils, tissue infiltration, and organ damage. In a subset of CEL patients, the FIP1L1/PDGFRA (F/P) oncoprotein is detectable. F/P exhibits constitutive tyrosine kinase activity and has been described to activate a number of signaling pathways. So far, however, little is known about F/P-dependent expression of proteins in leukemic cells and their potential role in the pathogenesis of CEL.**Aims**The aim of our study was to identify F/P-dependent cytokines and to investigate their potential functional role in the pathogenesis of CEL.**Methods**To screen for F/P-dependent cytokines, F/P was lentivirally expressed in growth factor-dependent TF-1 and Mo7e cells. In addition, Ba/F3 cells with doxycycline-inducible expression of F/P were generated. The human F/P+ cell line EOL-1 was used to study F/P-dependent signaling in neoplastic eosinophils. Expression of OSM in the bone marrow of CEL patients was investigated by immunohistochemistry and real time PCR.**Results**We found that F/P upregulates expression of oncostatin M (OSM), an IL-6 family cytokine, in various cell line models including F/P+ EOL-1 cells via activation of STAT5. Immunohistochemistry revealed that EMBP+ neoplastic eosinophils in the bone marrow of patients with CEL express substantial amounts of the OSM protein. Correspondingly, OSM mRNA levels were increased in the bone marrow of CEL patients compared to non-leukemic control samples. OSM secreted by F/P+ cells stimulated growth of fibroblasts and microvascular endothelial cells and upregulated production of angiogenic and profibrogenic cytokines in fibroblasts. Importantly, OSM derived from F/P+ neoplastic cells was found to stimulate production of SDF-1 in human fibroblasts. SDF-1 itself induced migration of EOL-1 cells in a dose-dependent manner.**Conclusion**Our data show that neoplastic cells in CEL express OSM in a F/P- and STAT5-dependent manner. F/P-mediated upregulation of OSM may contribute to microenvironment alterations as well as to taxis of neoplastic eosinophils into tissues suggesting that OSM might represent a new potential target in CEL.

0903

THE HDACI GIVINOSTAT MODULATES HEMATOPOIETIC TRANSCRIPTION FACTORS IN JAK2V617F CELLS FROM MYELOPROLIFERATIVE NEOPLASM PATIENTS

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Background. The HDAC inhibitor Givinostat (GVS) is a hydroxamate pan-HDAC inhibitor, and recently has been discovered to inhibit colony formation of cells from myeloproliferativeneoplasms (MPN) patients at doses at least 2-3 times lower than those required to affect cells bearing wild type JAK2. The mutant protein, confers to hematopoietic progenitors cells growth factor independent proliferation and colony formation.**Aims.** Analyse the biological effects of low and high doses of GVS on $JAK2^{V617F}$ mutated cells in long and short terms assays. Study the mechanism of action of GVS in $JAK2^{V617F}$ cells using gene expression profiling (GEP). **Methods.** Clonogenic assay, cytotoxicity, proliferation, cell death and erythroid differentiation was used for evaluate the biological effects of GVS in cell lines and CD34+ cell of patients with MPN. GEP was performed to obtain a global picture of the effect to GVS in HEL and UKE1 cell lines. Western Blot, Real-Time PCR (RTQ-PCR) and Chromatin Immunoprecipitation (ChIP) were performed to evaluate the molecular effects of GVS in cell lines.**Results.** Our study confirmed the higher sensitivity of mutated $JAK2^{V617F}$ with respect to unmutated MPN cell lines in colony and alamar blue assays. Furthermore we have confirmed that GVS inhibits JAK2 and STAT5 phosphorylation in mutated but not unmutated cell lines. By global gene expression analysis, we observed that GVS modulated 293 common genes in the $JAK2^{V617F}$ cell lines HEL and UKE1, of which 19 are implicated in cell cycle regulation and 33 in hematopoiesis. In particular the hematopoietic transcription factors NFE2 and C-MYB were downmodulated by the drug specifically in $JAK2^{V617F}$ cells at both the RNA and protein level. GVS also inhibited JAK2-STAT5-ERK1/2 phosphorylation, but modulation of NFE2 and C-MYB was JAK2-independent, as shown using the JAK2 inhibitor TG101209. Indeed GVS had a direct effect on the NFE2 promoters, as demonstrated by specific enrichment of their associated histone H3 acetylated at Lysine 9. Modulation by GVS of NFE2 was also observed in freshly isolated $CD34^+$ cells from MPN patients, and was accompanied by inhibition of their proliferation and differentiation towards the erythroid lineage.**Conclusions:** GVS inhibits the JAK2/STAT5/ERK1/2 pathway and independently expression of crucial hematopoietic TF, most importantly NFE2. These effects offer an explanation for the response of $JAK2^{V617F}$ cells to the drug, making this compound of particular interest in the context of MPN treatment.**Keywords:** Givinostat, $JAK2^{V617F}$, NFE2, C-MYB

0904

IN VITRO AND IN VIVO DATA CONFIRMED JAK1 GENE EXPRESSION AS PREDICTOR MARKER OF RESPONSE TO IFN IN ESSENTIAL THROMBOCYTHEMIA (ET)

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Background. Interferon-alpha (IFN) has shown antitumor and immunomodulatory activity in ET and has been associated with normalization of peripheral blood count and suppression of abnormal hematopoietic clone. Although about 80% of patients respond to this therapy, some patients could be defined as bad responders.IFN-α receptor lack tyrosine-kinase activity and rely on Janus-kinase, Tyk2 and Jak1, for their phosphorylation, on signal transducing proteins, such as STATs, for the transmission of intracellular messages. The STATs activated in response to IFNs include STAT1, STAT2, STAT3 and STAT5.STATs proteins induce the transcription of SOCSs, whose role is to extinguish cytokine signaling by inhibition of JAK kinase-activity directly through the KIR-domain, and indirectly promoting the proteasomal degradation of Jak2, by SOCS-box-motif.IFN, inducing SOCSs expression, inhibits TPO mediated signaling through Jak2 inhibition.In vitro studies suggest that clonal MPN progenitors may be more sensitive to IFN than their normal counterparts.**Aims.** This study aimed to identify molecular markers to predict IFN treatment responsiveness in ET patients. We evaluated the mRNA expression of JAK1, TYK2, STAT1, STAT3, SOCS1 and SOCS3, which signal cross-talks with the JAK-STAT pathway under IFN and TPO. **Methods.** We analyzed 46 ET patients treated with 3 million units of IFN-α-2b 5 times a week as induction (3 months), and 3 times a week as maintenance.Two groups of response were identified: *Responders(R)* (n=31), who achieved a reduction of platelet count below $400x10^9$/L, and *No-Responders(NR)* (n=15) who failed.For in vitro study, HEL and SET2 cell lines, carrying respectively homo and hemizygous $JAK2^{V617F}$ mutations, were treated with dose escalation of IFN (10-10000 IU/mL). Proliferation rate and apoptosis were evaluated after 24-48-72-96-168 hrs. The expression of target mRNA was explored in bone marrow samples of ET patients by RTq-PCR. Data were normalized as following: [mRNA normalized copy number (NCN)=mRNA target gene/mRNA GUSB*104]. **Results.** We detected JAK2 V617F mutation in 40% of R and 66% of NR. Patients showed a median spleen volume of 500 ml in R and 300 ml in NR group (p=0.04). No other clinical characteristics were different between the two patient categories.Among all six investigated target mRNA, we detected a significant higher JAK1 mRNA expression in NR than R (141638 vs 55116, respectively;p=0.000016). This enforces our previous data. Also, NR showed a higher expression of SOCS3 mRNA than R (15214 vs 10828, respectively;p=0.02). Thus, we confirmed our in vivo data also in an in vitro model of MPN.Indeed, SET2 cell line shows a significant inhibition of cell growth (p=0.007) and apoptosis induction (p=0.005) upon IFN treatment compared with HEL cell line. Molecular analysis revealed that JAK1 and SOCS3 mRNA expression was higher in HEL cell line than in the IFN sensible SET cell line (p=0.009, p=0.01 respectively). **Conclusions.** In vitro and in vivo data confirmed JAK1 gene expression as predictor marker of response to IFN in ET patients. Patients with low levels of JAK1 and SOCS3 mRNA may be eligible for IFN therapy. Moreover, patients with IFN-unresponsiveness molecular signature may be considered for JAK1/JAK2 inhibitor treatment.

0905

HIGH FREQUENCY OF MPL MUTATIONS AMONG PATIENTS WITH JAK2V617F-NEGATIVE ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS SELECTED BY THE PRESENCE OF ENDOGENOUS MEGAKARYOCYTIC COLONY

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Background. Spontaneous hematopoietic colony formation from bone marrow or peripheral blood was shown to be a functional hallmark of deregulated signalling pathway in myeloproliferative neoplasms (MPNs). Consequently, it is used as a diagnostic criteria according to the 2008 World Health Organization (WHO) classification but is limited to the study of erythroid colony in polycythemia vera. Endogenous megakaryocytic colony (EMC) growth, even if not included in WHO criteria, has also proven to be a good diagnostic tool in MPNs especially in essential thrombocythemia (ET). Considering involvement of megakaryocyte lineage in MPNs, EMC obtained from bone marrow with standardized and reproducible method, gives phenotypic argument for MPNs clonality and information complementary to *JAK2V617F* status. **Aims**. The current place of *in vitro* EMC in ET and primary myelofibrosis (PMF) diagnosis needs to be evaluated in particular in comparison to genetic abnormalities (*JAK2V617F*, *MPL* mutations). **Methods.** In this four-centre retrospective study, we compared endogenous erythroid colony (EEC) and EMC formation with *JAK2V617F* status in 190 ET and 15 PMF, diagnosed between January 1st, 2007 and December 31th, 2010. EEC and EMC were assessed in peripheral blood and/or bone marrow using a standardized method. *JAK2V617F* and *MPL* mutations were detected using ARMS-PCR (Amplification Refractory Mutation System) and HRM (High Resolution Melt) respectively. Results. In PMF, 53% of patients (8/15) had *JAK2V617F* mutation and 60% (9/15) led to spontaneous hematopoietic colony formation (EEC and/or EMC). In ET, 82% of patients (155/190) displayed EEC and/or EMC growth. Compared with *JAK2V617F* status (68% of ET (129/190)), the study of spontaneous colony formation significantly improves the sensitivity for diagnosis of ET (Mac Nemar's test, $p<0.05$). Moreover, we used the presence of EMC to select a subgroup of 43 patients among JAK2-negative ET/PMF. Surprisingly, somatic mutations of MPL (thrombopoietin receptor) were detected in 16 out of 43 (37%). This result contrasts dramatically with the 3% and 10% *MPL* mutations frequencies usually described in ET and PMF respectively. **Conclusions**. Our study shows that EMC associated with EEC assays improved sensitivity of clonogenic cultures for the diagnosis of ET and PMF. Moreover, EMC might serves as a predictive tool for the selection of potent *MPL* mutants among JAK2-negative ET and PMF. Altogether, these data suggest that EMC formation should be consider as a useful parameter for the phenotypic and genotypic characterizations of MPNs.

0906

EASY AND RAPID QUANTIFICATION OF JAK2 V617F MUTATION BY NEXT GENERATION SEQUENCING

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Background. Since the discovery of JAK2 V617F mutation, the quantification of mutant allele has been widely studied especially in order to predict clinical outcome but also to better understand the role of V617F in myeloproliferative neoplasms. Some laboratories have developed JAK2 V617F quantification technique based on real time quantitative PCR with variable sensitivities and specificities. In order to compare these techniques, some groups including the European Leukemia Net have organized Quality Control rounds evaluations. The recent conclusions are very promising but require very stable standards and cooperation with manufacturers. NGS is a very sensitive technique that allows absolute quantification without standard curve and is applicable for many targets. **Aims**.To evaluate the feasibility and the performance of JAK2 V617F quantification by NGS.MethodsQuantification was performed on genomic DNA using the Ion Torrent platform. Primers were designed in order to produce a short PCR product (81bp) including the V617F mutation in the median part. The standard barcoded amplicon libraries with ligation of adapter and template preparation were used. The sequencing was performed on 314 chips.Samples used were UKE1 cell line, 4 negative controls collected from healthy subject, 11 patients quantified by the Mutaquant kit from Ipsogen (mutated allele percentage ranging from 94% to 1.3%), and a two-fold dilution. The dilution (100% to 0.024%) of the UKE1 cell line was performed in negative control sample B. Each diluted sample was further processed with multiple barcodes to assess reliability of libraries construction.The allelic ratio was computed as the number of reads with V617F mutation versus the total number of reads. Only reads with at least 80% of bases with Q17 qualities were taken into account. The probability that allelic ratio was positive was computed with Fisher exact test. **Results**. The normal samples showed only one read with V617F mutation. Combining the 4 samples, the computed allelic ratio was about 0.0045%. The computed allelic ratios from the diluted UKE1 show a straight linear correlation with theoretical dilution (r=0.983 pearson correlation, p-value=1.100e-08). All diluted samples but one are below the theoretical values. Last but not least, all diluted samples showed positive allelic ratio values with p-value < 2.5^{-5}. Patient samples with mutaquant allelic ratio ranging from 94% to 1.3% showed linear correlation (r=0.974 pearson correlation, p-value=4.003e-07).Summary/conclusionsWe report detection values below 10^{-4} with p-value under 2.5^{-5}. There is no absolute limitation in low allelic ratio detection as we can always sequence more reads. The detection limit is determined by the false positive in normal samples. The cost is increasingly low as our data show that we can reliably compute allelic ratio from 20000 reads to reach 5.10^{-4}. The 314 chip allows to multiplex from 10 to 15 samples by run. We are planning to cross check more patient blind-quantified from others centers and to enable detection reaching 10^{-5} on diluted samples. The use of Ion Torrent technologies is a very easy and simple way to quantify JAK2 V617F mutation.

0907

A N,N'-DIARYLUREA INHIBITOR OF PROTEIN TRANSLATION INITIATION SELECTIVELY SUPPRESSES THE PROLIFERATION OF CD34+ CELLS ISOLATED FROM POLYCYTHEMIA VERA PATIENTS VIA ACTIVATION OF THE HRI-EIF2α PATHWAY

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Background. Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) comprise the subgroup of BCR-ABL negative myeloproliferative neoplasms (MPN). Identification of the gain-of-function $JAK2^{V617F}$ mutation in over 95% of PV and approximately 50-60% of ET and PMF patients has greatly advanced our understanding of MPNs. MPNs are associated with significant morbidity, reduced quality of life, and premature death. In addition to phlebotomy, treatment with hydroxyurea or interferon-alpha remains standard of care in PV patients. Although these interventions help control symptomatic disease, they have little if any effect on JAK2 allelic burden and do not alter disease natural history. Although JAK2-inhibitors are approved for PMF, their role in PV remains to be defined, and novel therapies are desperately needed for patients. **Aims.** We investigated the efficacy of *N,N'*-diarylurea compounds, previously shown to inhibit solid tumor cell growth by reducing the eIF2-GTP-$tRNA_i^{Met}$ translation initiation complex, in decreasing the proliferative capacity of $JAK2^{V617F}$ mutant cells. We demonstrate that a specific compound, #1781, selectively reduces the proliferation of both human cell lines (HEL and SET-2) and primary CD34+ cells isolated from PV patients without altering growth characteristics of normal CD34+ cells. **Methods.** Cell growth and apoptosis were measured by CellTiter Blue cell proliferation assay and Annexin V staining, respectively. Protein phosphorylation and expression levels were determined by immunoblotting, and mRNA expression determined by quantitative real-time PCR. FACS was used to sort transfected cells and to detect cell surface marker expression. Semisolid methylcellulose culture was used to evaluate BFU-E formation in human CD34+ cells.**Results.** We examined the effects of several protein translational inhibitors on proliferation of human $JAK2^{V617F}$-mutant cell lines [HEL (homozygous), and SET-2 (heterozygous)], and normal human CD34+ cells. We found that a novel *N,N'*-diarylurea compound (#1781) potently decreased HEL and SET-2 cell proliferation at doses non-toxic to normal human CD34+ cells, exhibiting significantly lower IC_{50}

values in both HEL (4 μM)] and SET-2 (3 μM)] cells compared to normal CD34+ cells (> 10 μM)]. #1781 treatment increased eIF2α phosphorylation, while replacement of endogenous eIF2α with non-phosphorylated eIF2α^{S51A} rendered HEL cells less sensitive to #1781. Furthermore, knock-down of heme-regulated inhibitor kinase (HRI), which phosphorylates eIF2α, made cells less sensitive to #1781, indicating that #1781 mediates cell growth inhibitory effects by activating the HRI-eIF2α pathway, thereby inhibiting protein translation initiation. #1781 treatment significantly reduced phospho-JAK2, phospho-STAT5, phospho-ERK1/2 and c-Myc expression within 24 hours. Finally, #1781 treatment reduced BFU-E colony numbers in CD34+ cells isolated from four PV patients by 49% to near baseline, without adversely affecting BFU-E formation in normal CD34+ cells ($p<0.02$, t-test), indicating that #1781 selectively suppresses PV-associated erythropoiesis. In contrast, an inactive analog (#1527) of #1781 exhibited no effects on cell growth or signaling. **Summar and Conclusions**. Our study demonstrates that a novel *N,N'*-diarylurea small-molecule protein translational inhibitor suppresses proliferation of human PV hematopoietic progenitors via selective inhibition of protein translation and signal transduction pathways required for PV cell growth and survival. Our results provide important pre-clinical data supporting further study of this class of compounds as therapy for MPN patients.

0908

INCREASING THE SENSITIVITY OF JAK2V617F DETECTION MUST BE CONSIDERED CAUTIOUSLY FOR ACCURATE DIAGNOSIS

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Background. According to the WHO classification, the detection of the JAK2V617F mutation in Myeloproliferative neoplasms (MPN) diagnosis is one major criteria for Polycythemia Vera (PV), Essential Thrombocytemia (ET) or Myelofibrosis (MF) diagnosis. Stretching down the lower limit of detectability of a molecular marker is of utmost importance for accurate diagnosis but also for its potential use as minimal residual disease marker.. However, using methods with higher sensitivity at the time of diagnosis may lead to the identification of patients bearing very low mutant allele burden with questionable clinical relevance. **Aims**. To explore whether changing for a more sensitive method may improve the diagnosis in Myeloproliferative Neoplasms (MPN) and identify potential pitfalls. **Methods.** Results of JAK2V617F screening from our lab were analyzed 6 months after changing from the JAK2 Mutascreen® (Ipsogen) method (sensitivity: 2%) for the JAK2 Mutaquant® (Ipsogen) method (sensitivity: 0.1%). Absence of false positive results was confirmed on 49 samples taken from blood donors and the level of sensitivity was established on serial dilutions of mutated patient's DNA. **Results.** We re-tested with the Mutaquant (MQ) method 191 patients previously analyzed using the Mutascreen (MS) method. 98 patients had been found positive and 93 negative with MS. In 98/98 cases the MQ assay confirmed the positivity found previously using the MS assay. In contrast, 7 patients among the 93 (7.5%) previously found negative by the MS assay were found positive with MQ. The allele burden for these 7 patients was always lower than 1% (median 0.49%, range 0.1% to 1%). In 3/7 patients, additional sample (taken 1 to 5 years before) were reanalyzed using MQ method: all these samples were confirmed positive with a similar JAK2V617F allele burden. In 5 patients the diagnosis of MPN could be made according to the WHO criteria including JAK2V617F mutation as a major criterion: 3 with ET, 1 with PV, 1 with MF. One patient had unclassifiable MPN with hyperleucocytosis, myelemia and trisomy 8. Finally 1 patient presented with repeated pulmonary embolisms with normal blood counts (Hct: 47.4%; Hgb: 17.1 g/dl; Red Cell Mass: +20% of the expected value; WBC: 7.7 G/L; Plt: 335 G/L). In this latter case, the mutation was already present at the same level 5 years before. The presence of an additional *MPL* or *JAK2*-exon 12 mutation was excluded in these patients. **Summary and Comments.** This study argues for the choice of a *JAK2V617F*detection method characterized by a detection limit under 1% as no indication is given regarding the allele burden according to the recent WHO classification's major criteria. In this regard, patients detected with a mutant allele burden of 0.5% should be considered equally to those with 5% or 50%. However as shown for 1 out of 7 patients, the correlation between the presence of the mutation and a possible MPN may be questionable. The decision for any potential therapeutic intervention must be made very cautiously in these cases until prospective clinical studies are available.

0909

SPECIFIC INHIBITION OF CHOLESTEROL SYNTHESIS INDUCES CYTOPROTECTIVE AUTOPHAGY IN HUMAN LEUKEMIC CELLS BUT NOT NORMAL HUMAN LYMPHOCYTES

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Background. In addition to cholesterol lowering effects, high dose statin treatments exhibit anti-leukemic properties *in vitro.* These effects are due to suppression of the mevalonate pathway by the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A. Apart from being involved in cholesterol synthesis, the mevalonate pathway is also involved in the synthesis of farnesylated and geranylgeranylated proteins which are critical to key cellular functions such as cell survival, proliferation, and growth. Furthermore, inhibition of cholesterol synthesis, protein farnesylation and geranylgeranylation have all been shown to have independent antileukemic effects. **Aims**. Our aims was to discern the effects of low dose statin treatments on leukemias as well as to determine whether these effects were due to the inhibition of cholesterol synthesis, or the inhibition of protein farnesylation or geranylgeranylation. **Methods.** The human leukemic cell lines HL-60, REH, JVM-2 and JVM13, as well as PBMCs isolated by density centrifugation from 6 leukemic patients and 3 healthy volunteers were used. Cell viability was determined by acid phosphatase assay. Apoptotic responses were analyzed by Western blotting for active caspase-3, and by flow cytometry using annexin V-FITC and propidium iodide for phosphatidylserine externalization and DNA fragmentation. Autophagy was determined by electron and fluorescence microscopy as well as by Western blotting for the appearance of the LC3II variant of the LC3 protein. Inhibition of autophagy was performed by use of bafilomycin A1 and by knockdown of the beclin-1 protein using siRNA techniques.

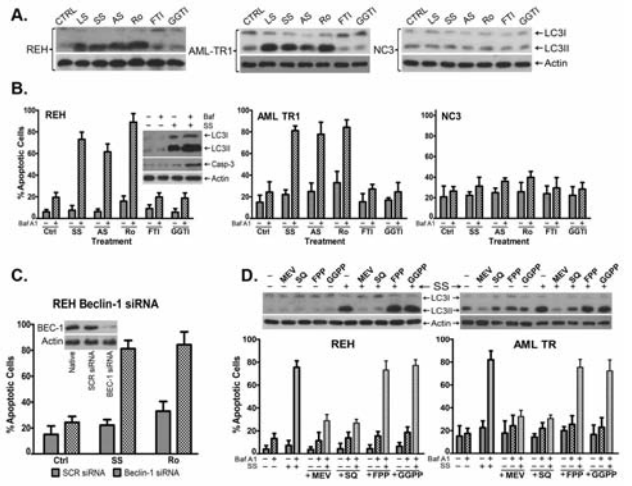

Figure 1. Specific Inhibition of Cholesterol Synthesis Induces Cytoprotective Autophagy In Human Leukemic Cells But Not Normal Human Lymphocytes.

Results. Lovastatin, simvastatin and atorvastatin treatment, as well as specific inhibition of cholesterol synthesis, farnesylation or geranylgeranylation all resulted in dose-dependent decreases in the viabilities of leukemic cell lines and leukemic patients' PBMCs. Flow cytometric analysis of cell cycle phase distributions and apoptosis suggested that the statin-induced decrease in cell viability was due to inhibition of cell cycle progression as well as apoptosis. These effects were dramatically muted in normal human lymphocytes. Significantly, we have shown that the inhibition of the mevalonate pathway by statins, as well as specific inhibition of cholesterol synthesis Ro-48-8071, but not the inhibition of protein farnesylation or geranylgeranylation, induces a strong autophagic response in leukemic cells but not normal human lymphocytes. Furthermore, inhibition of autophagy by treatment with the vacuolar acidification inhibitor bafilomycin A1, or siRNA-mediated knockdown of the autophagy regulating

protein beclin-1, dramatically increases apoptosis in leukemic cells, an effect that is attenuated by the addition of mevalonate pathway product squalene, but not by the addition of farnesylpyrophosphate or geranylgeranylpyrophosphate. **Conclusions.** Our study shows that inhibition of the mevalonate pathway by low dose statin treatment induces cytoprotective autophagy that is dependent specifically on inhibition of cholesterol synthesis. The effect seems to be specific to leukemic cells, as it was not observed in normal human lymphocytes. Furthermore, we have shown that inhibition of autophagy uncovers a potent apoptosis-inducing effect of cholesterol synthesis inhibition in leukemic cells. These results suggest that selective cholesterol reducing therapies in combination with autophagic inhibition warrant further exploration as potential candidates for selective anti-leukemic therapy.

0910

PHOSPHATIDYLINOSITOL-3 KINASE AND NF-κB AS POTENTIAL THERAPEUTIC TARGETS IN MYELOID MALIGNANCIES ASSOCIATED WITH HYPEREOSINOPHILIA AND PDGF RECEPTOR REARRANGEMENTS

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Background. ETV6-PDGFRB and FIP1L1-PDGFRA are receptor-tyrosine kinase fusion genes causing chronic myeloid malignancies associated with hypereosinophilia. Imatinib is the standard treatment of this disease but resistance has been reported.The **aim** of this work was to set up a human model for this disease and analyze the mechanisms whereby PDGF receptor fusions induce hypereosinophilia in order to identify new therapeutic targets. **Methods.** We introduced ETV6-PDGFRB and FIP1L1-PDGFRA using lentiviral particles in human CD34$^+$ hematopoietic progenitors and stem cells isolated from umbilical cord blood. Signal transduction and gene regulation were explored by flow cytometry and Affymetrix microarrays. **Results.** We first analyzed the expression of PDGFRA in CD34+ cells. By contrast to previous reports, no PDGFRA expression (protein of mRNA) could be detected on these cells. CD34+ cells were then transduced with ETV6-PDGFRB and FIP1L1-PDGFRA. These cells formed hematopoietic colonies even in the absence of cytokine. Both oncogenes also stimulated the proliferation of cells in liquid culture and their differentiation into eosinophils. This model thus recapitulated key features of the myeloid neoplasms induced by ETV6-PDGFRB and FIP1L1-PDGFRA. Microarrays were used to identify genes regulated by these oncogenes. A bioinformatics analysis based on TFactS revealed the regulation of a number of transcription factors. By flow cytometry, we confirmed that both fusion genes activated the transcription factors STAT1, STAT3, STAT5 and NF-κB. The importance of STAT5 downstream both oncogenes is well established. We focused on NF-κB activation, whose importance in PDGF signal transduction has been debated. Phosphatidylinositol-3 kinase inhibition blocked NF-κB activation in transduced progenitors and patient cells. NF-κB was also activated in the human FIP1L1-PDGFRA-positive leukemia cell line EOL1, the proliferation of which was blocked by the proteasome inhibitor bortezomib and the IkB kinase inhibitor BMS-345541. A mutant IκB that prevents NF-κB nuclear translocation inhibited cell growth and the expression of eosinophil markers, such as the interleukin-5 receptor and eosinophil peroxidase, in progenitors transduced with ETV6-PDGFRB. **Conclusions.** We show that human CD34$^+$ cells expressing PDGF receptor fusion oncogenes proliferate autonomously and differentiate towards the eosinophil lineage in a process that requires a phosphatidylinositol-3 kinase - IKK - NF-κB pathway. These results suggest new treatment possibilities for imatinib-resistant myeloid neoplasms associated with PDGF receptor mutations.

0911

AP-1 IMPACTS ON ANGIOGENESIS IN BCR/ABL DRIVEN DISEASE

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Background. Several forms of leukemia are caused by chromosomal translocation resulting in the Philadelphia chromosome. The resulting fusion product is the constitutively active BCR/ABL tyrosine kinase that activates several signalling pathways. One endpoint of these signalling cascades is the AP-1 transcription-factor family which contains important modulators of transformation and tumorigenesis. Our laboratory has investigated the effects of JUNB and c-JUN for BCR/ABL driven leukemogenesis. Whereas JUNB suppresses BCR/ABL driven leukemia and is considered a tumor suppressor for this disease entity c-JUN is promoting tumor formation and accelerates leukemogenesis. Accordingly, JUNB is lost in BCR/ABL transformed cells in people. **Aims.** In this project we aim to elucidate the mechanism how c-JUN contributes to leukemia formation. We intend to characterize the effects of c-JUN on angiogenesis and its relevance in BCR/ABL driven malignancies. Several AP-1 target genes have been identified, that are key players in angiogenesis. We intend to characterize their expression and role for BCR/ABL driven disease. **Methods.** We use transgenic mouse models to elucidate the role of c-JUN in BCR/ABL driven disease. Moreover we have established several cell lines lacking components of the AP-1 family to analyse gene expression. **Results**. We have recently delineated a novel mechanism how the AP-1 transcription factor c-JUN regulates lymphoma formation in Bcr/Abl+ cells. During the course of these experiments we learnt that c-JUN is involved in the regulation of de novo blood vessel formation in BCR/ABL driven disease. Angiogenesis was severely impaired if the tumor cells lack the expression of c-JUN (c-JUNΔ/Δ). In accordance, the expression of VEGF-A and VEGF-C was significantly decreased in c-JUNΔ/Δ lymphoma cells. According to the opposing roles of c-JUN and JUNB we also found that JUNB deficient cells show an increased VEGF-A expression. An angiogenesis array revealed a consistent pattern of differentially expressed pro- and anti-angiogenetic genes in Bcr/Abl+ cells lacking c-JUN compared to wt. Among these genes, we have already confirmed the upregulation of TGFβ3 in c-JUNΔ/Δ cells. TGFβ3 is considered to be an important suppressor of tumor angiogenesis. **Conclusions.** We here propose that c-JUN not only regulates cell proliferation but also angiogenesis in Bcr/Abl+ cells by regulating a number of different genes. We propose that this regulation impacts on leukemogenesis.Our study has human relevance as alterations in the AP-1 transcription factor c-JUN are common in human cancer patients. It is thus essential to understand the molecular mechanisms driven and maintained by the AP-1 transcription factor family.

0912

IRON-DEPENDENT REGULATION OF HEPATIC HEPCIDIN EXPRESSION IN HJV-/- MICE

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Hemojuvelin (HJV) is a bone morphogenetic protein (BMP) co-receptor that plays a pivotal role in systemic iron homeostasis. Mutations in HJV are causatively linked to juvenile hemochromatosis, an early onset and severe form of hereditary iron overload. The marked suppression of hepcidin expression observed in juvenile hemochromatosis patients and in HJV-/- mice, suggests a function of hemojuvelin, as an upstream regulator of this liver-derived iron-regulatory peptide hormone. To elucidate the role of HJV in iron-sensing pathways, we employed HJV-/- mice and analyzed their responses to dietary or other iron manipulations.8-weeks old HJV-/- and wild type (HJV+/+) control mice were placed in diets of varying iron content (low, normal and high) for 4 weeks. Serum iron parameters, liver iron deposition and hepatic hepcidin, pSmad1/5/8, pErk1/2, BMP6, Smad7 and Id1 expression were assessed.Serum iron levels were significantly increased in HJV-/- mice in comparison to wild type controls in all diet regimens. Interestingly, transferrin was highly saturated (97%) in HJV-/- mice regardless the iron content of the diet. Massive liver iron deposition in HJV-/- mice was assessed with both a qualitative (Prussian blue stain) and quantitative (ferrozine assay) technique. As expected, hepatic levels of hepcidin mRNA correlated to dietary iron intake in wild type mice. Surprisingly, hepcidin mRNA expression was likewiseresponsive to dietary iron intake in HJV-/- mice, despite the marked overall suppression of hepcidin mRNA levels as compared to wild type controls. HJV-/- mice placed in low and normal iron diets exhibited significantly higher levels of BMP6 mRNA compared to wild type controls.Our data are consistent with earlier observations that the disruption of HJV leads to suppression of hepcidin expression and severe iron overload. Furthermore, our data suggest that HJV is not essential for iron sensing and rather acts as an enhancer for hepcidin expression. Importantly, our findings uncouple the iron-dependent regulation of hepcidin expression from alterations in transferrin saturation, at least in HJV-/- mice.

0913

REPROGRAMMING OF THE STAT SIGNALING NETWORK IN CD4+ CELLS: A NOVEL IMMUNOMODULATORY ROLE OF 5-AZACITIDINE

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Background and Aims. Signal transducer and activator of transcription (STAT) proteins have essential roles in the epigenetic control of TH cell differentiation and defective STAT signaling can compromise tumor immunity. 5-azacytidine has immunomodulatory properties which may contribute to its antitumor activity. Though the exact mechanism is unknown, it has been shown that the demethylation of regulatory elements of transcription factors involved in T helper (TH) cell polarization may enhance tumor immunity by restoring the deficient cytokine signaling. Myelodysplastic syndromes (MDS) have a strong immunopathogenetic component, but the CD4+ cell signaling via STATs has not yet been intensively investigated.

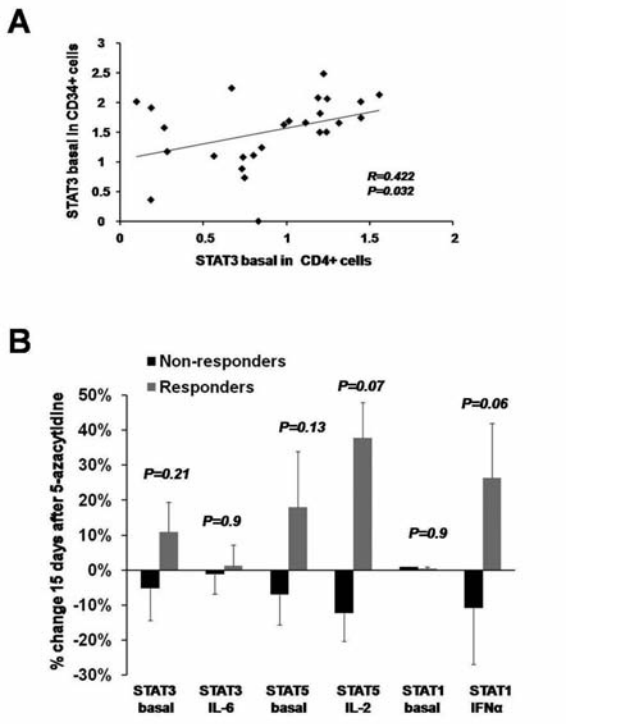

Figure 1. Correlation of basal STAT3 levels in CD4+ and CD34+ cells (A). Alterations of signaling nodes 15 days after 5-azacitidine initiation (B).

We applied phospho-specific flow cytometry to explore the alterations of STAT signaling in CD4+ cells during 5-azacitidine treatment and addressed their association with clinical and biological parameters.METHODSPeripheral blood (PB) and bone marrow (BM) mononuclear cells of 31 high risk MDS patients were obtained before and 15 days after 5-azacitidine initiation. According to WHO classification, 15.5% of the patients had RAEB-I, 40.5% RAEB-II, 19% CMML-II and 25% AML/MDS. Cytogenetics according to IPSS were good in 39% of the patients, intermediate in 39% and poor in 22%. Based on the IWG response criteria patients were divided into responders (CR and PR, n=9) and non-responders (stable disease and failure, n=22). Cells were either left untreated (basal levels) or stimulated with IL-6, IL2 and IFNα for 15' (potentiated response) and then stained for surface CD4, CD3, CD34 and intracellularly with phospho-STAT1, 3 and 5. The following potentiated signaling nodes, i.e. target/stimuli combinations, were studied: STAT3/IL-6, STAT5/IL-2 and STAT1/IFNα. Basal levels were expressed as log2 [Median fluorescence intensity (MFI) (unmodulated)/MFI (isotype)] and potentiated nodes as log2 [MFI (modulated)/MFI (unmodulated)]. Statistical comparisons were performed by unpaired Student's t test or one-way ANOVA as appropriate. Correlations were assessed by Spearman's rank test. **Results.** No differences were observed in both basal and potentiated nodes between the various MDS subtypes or in relation to the cytogenetic status. By contrast, non-responders had significantly higher basal STAT3 levels in both PB (p=0.04) and BM (p=0.02) CD4+ cells, whereas the other nodes were similar among the two groups. Interestingly, basal STAT3 levels were also higher in CD34+ blasts of the same patients and correlated positively with constitutive STAT3 expression of CD4+ cells (R=0.422, p=0.032) Figure 1A), indicating an underlying mechanism of global upregulation of STAT3 in patients refractory to 5-azacitidine. More important, all nodes, except basal STAT1, were upregulated 15 days after treatment initiation in responders, in contrast to non-responders where the same nodes were slightly dowregulated. However, the differences were marginally significant only in STAT5/IL-2 (p=0.07) and STAT1/IFNα (p=0.06) nodes (Figure 1B).**Conclusions**. STAT3 is constitutively overexpressed in both CD4+ and CD34+ cells in MDS patients resistant to 5-azacitidine, illustrating the multiple roles of STAT3 in tumorigenesis. Also, the upregulation of signaling nodes after 5-azacitidine treatment only in responding patients indicates enhancement of CD4+ cell cytokine responsiveness by epigenetic reprogramming of STAT signaling network, which may potentially favor the antitumor response.

0914

CHARACTERIZATION OF CYCLIN E EXPRESSION IN MULTIPLE MYELOMA AND ITS FUNCTIONAL ROLE IN SELICICLIB-INDUCED APOPTOTIC CELL DEATH

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Background. Multiple Myeloma (MM) is a lymphatic neoplasm characterized by clonal proliferation of malignant plasma cell that eventually develops resistance to chemotherapy. Drug resistance, differentiation block and increased survival of the MM tumor cells result from high genomic instability. Chromosomal translocations, the most common genomic alterations in MM, lead to dysregulation of cyclin D, a regulatory protein that governs the activation of key cell cycle regulator - cyclin dependent kinase (CDK). Genomic instability was reported to be affected by over expression of another CDK regulator - cyclin E (CCNE). This occurs early in tumorgenesis in various lymphatic malignancies includingCLL,NHL and HL. We therefore, sought to investigate the role of cyclin E in MM. **Results.** CCNE1 expression was found to be heterogeneous in various MM cell lines. Incubation of MM cell lines with seliciclib, a selective CDK-inhibitor, results in apoptosis which is accompanied by down regulation of MCL1 and p27 protein levels. In addition, seliciclib treatment significantly inhibited the phosphorylation of MCL1 and CCNE1 in MM cells. Ectopic over expression ofCCNE1 resulted in reduced sensitivity of the MM tumor cells in comparison to the paternal cell line, whereas CCNE1 silencing with siRNA increased the cell sensitivity to seliciclib. Adhesion to FN of MM cells was prevented by seliciclib, eliminating adhesion-mediated drug resistance (CAM-DR) of MM cells. Furthermore, combination of seliciclib with doxorubicin, a known drug that displays CAM-DR, significantly enhanced anti-MM effect. To achieve more prominent CDK inhibition, we evaluated the effect of seliciclib in combination with another CDK inhibitor, flavopiridol. Combined treatment with seliciclib and flavopiridol demonstrated enhanced anti-myeloma activity, effectively reduced CCNE1 and CCND1 protein levels, increased subG1 apoptotic fraction and promoted MM cell death in BMSCs co-culture conditions, therefore over-coming stroma-mediated protection. **Conclusions**. We suggest that CDK combined inhibition with seliciclib effectively targets MM and overcomes microenvironment-produced resistance. CCNE1 is involved in seliciclib-induced apoptosis and may affect the sensitivity of MM cells to the treatment. All together, our data provide the rationale for CDK inhibition as part of therapeutic strategy in myeloma patients.

0915

TARGETING NEUROPILIN-1 IN CLL AND MULTIPLE MYELOMA

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B-cell chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world with an incidence of 2-6 cases per 100,000 people per year. It is characterized by a clonal growth of long lived, slowly proliferating mature CD19+, CD5+, CD23+ B lymphoid cells in the bone marrow (BM), peripheral blood (PB) and lymphoid tissues. Multiple myeloma (MM) is a bone marrow-based, multifocal plasma cell neoplasm associated with a paraprotein in serum and/or urine. It comprises about 1% of malignant tumours, 10-15% of haematopoietic neo-

plasms and causes 20% of deaths from haematological malignancies. Standard therapies result in long durations of remission in most patients, while newer treatment regimens, which include the anti-angiogenic agents thalidomide and lenalidomide as well as the proteasome inhibitor, bortezomib, have improved outcomes. However, both diseases remain incurable and are associated with multiple disease relapses and death. Increased angiogenesis appears to play an important role in CLL and MM progression, VEGF emerging as the most clinically relevant of the known angiogenic factors. The transmembrane VEGF co-receptor Neuropilin-1 (NRP-1) plays an important role in angiogenesis and progression of many diseases, and it is expressed on endothelial and some tumour cells, including breast, lung and prostate. Previous studies have also shown NRP-1 acting through VEGFR-dependent and independent mechanisms. NRP-1 has been detected in CLL cells of approximately 65% of patients and elevated NRP-1 levels have also been found in bone marrow of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients compared to healthy controls. However, the importance of NRP-1 in hematological malignancies remains to be established.The aim of this study is to elucidate NRP-1 role in CLL and MM using primary cells isolated from CLL patients and a panel of MM cell lines. Western blot analysis show that NRP-1 and VEGF receptors are expressed in primary CLL cells and MM cell lines. CLL serum samples also express considerable levels of NRP-1. CLL cells treated with the NRP-1 specific ligand $VEGF_{165}$ increase expression of NRP-1 and the anti apoptotic protein Mcl-1 in a dose dependent manner, suggesting that NRP-1 signaling may play a role in CLL cell survival. NRP-1 knockdown using siRNA specific nucleotides results in decreased p42/p44 ERK activation, while Akt phosphorylation is unaffected. This suggests that NRP-1 signals predominantly through ERK in this system. Additionally, NRP-1 signaling blockade using a peptide corresponding to the NRP1 binding site on $VEGF_{165}$ in NRP-1-expressing MM cell lines decrease cell viability.These results suggest that targeting NRP-1 signaling may be valuable for CLL and MM treatment.

0916

C-MYC CONTROLS T CELL TRANSFERRIN RECEPTOR EXPRESSION DOWNSTREAM OF JAK AND GSK3 MEDIATED SIGNALLING PATHWAYS

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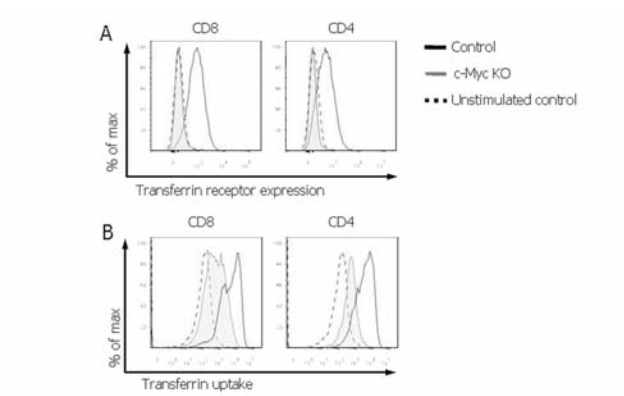
Background. Previous studies have shown that the proto-oncogene c-Myc is essential for cell growth and proliferation of both normal and transformed lymphocytes. It has also been recently been demonstrated that the expression of c-Myc in T cells is required for the metabolic reprogramming that accompanies T cell activation and in particular is necessary for glucose metabolism and glycolysis. What controls the expression of c-Myc in T cells and whether c-Myc expression is sufficient for metabolic reprogramming of T cells is unknown. **Aims.** We set out to identify signalling pathways that physiologically regulate c-Myc expression and to further identify important downstream c-Myc biological functions.



c-Myc is required for the increased transferrin receptor surface expression and transferrin uptake triggered by naïve T cell activation. Flow cytometric analysis of transferrin receptor expression (A) and transferrin uptake (B). Splenocytes from CD4-Cre/c-$Myc^{fl/fl}$ and litter mate control mice (Cre neg/c-$Myc^{fl/fl}$) were unstimulated or activated with CD3 (5µg/ml) and CD28 (3µg/ml) antibodies for 24 hours. All data are representative of 3 independent experiments.

Figure. c-Myc is required for the increased transferrin receptor surface expression and transferrin uptake triggered by naïve T cell activation.

Methods. We used specific inhibitors to probe the signalling pathways responsible for c-Myc regulation downstream of the T cell antigen receptor and the interleukin-2 receptor in primary T cells. To investigate c-Myc function we used both gene knockout and retroviral expression strategies. This allows us to probe the consequences of both failure to upregulate c-Myc on T cell activation and sustained c-Myc expression in the absence of cytokine signals. **Results.** We show that c-Myc expression in CD8 T cells is initiated in response to antigen receptor signals and then sustained by common gamma chain cytokine mediated activation of Janus kinase 3 (JAK3). Moreover, c-Myc protein levels in CD8 T cells are tightly controlled by GSK3 mediated signalling pathways that ensure c-Myc protein has a very short half life. Immune activated T cells thus cannot autonomously sustain c-Myc protein levels although they can sustain high levels of c-Myc mRNA; to maintain c-Myc protein expression there is a requirement for sustained common gamma chain cytokine mediated activation of JAK3. Analysis of c-Myc function revealed that c-Myc is required and sufficient to sustain transferrin receptor expression and transferrin uptake by T cells. This effect was selective as c-Myc expression was not sufficient to maintain glycolysis or amino acid uptake in T cells. Moreover, c-Myc expression also was insufficient to drive cell survival or protein synthesis and cell growth in primary non-transformed T cells. **Summary and Conclusions.** Herein we demonstrate that c-Myc expression in T cells is controlled by cytokine signals mediated through JAK3 and GSK3. Furthermore c-Myc expression is both necessary and required for the expression of the transferrin receptor and transferrin uptake. This ability of c-Myc to control transferrin uptake is important because iron is a critical co-factor for many key metabolic enzymes that control ATP generation. Work from other groups has suggested that targeting the transferrin receptor with monoclonal antibodies or using iron chelators may be a useful strategy in haematological malignancies. The current study provides a rationale for investigating this further in T cell malignancies.

0917

FLT3 MUTATION STATUS IN AML ALTERS THE TRANSCRIPTIONAL AND FUNCTIONAL ROLE OF CREB

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Background. In acute myeloid leukemia (AML) frequent mutations in signaling pathways are found. Some of these altered proteins are used to determine disease prognosis and treatment regime. An example is the receptor tyrosine kinase Flt3. Internal tandem duplications in Flt3 (Flt3-ITDs) changes its functionality and also changes downstream signaling and transcriptional activities. Moreover, Flt3-ITD is known as a high risk prognostic factor in AML. Additionally, activation or up-regulation of cAMP response element-binding protein (CREB) is found to be a bad prognostic factor in AML. Currently there is no evidence of a direct interaction between these two interesting members of important signaling pathways in AML.**Aims.** We aim to elucidate the potential connection between Flt3 and activation of CREB in AML. **Methods.** We stimulate Flt3 with its ligand (FL) and investigate the phospho-signaling cascade from Flt3 to CREB using phospho-specific flow cytometry. We have a panel of leukemia cell lines defined statuses of Flt3 or transduced cell lines overexpressing different forms of Flt3. Inhibitors of signaling pathways are used to verify the results. In order to elucidate the mechanisms behind FL regulation of CREB, we utilized a CREB promoter assay to enlighten the transcriptional differences between Flt3-wt and Flt3-ITD. The data obtained were verified by qPCR. In addition, we aim to verify the most important findings in a panel of primary AML cells with defined Flt3 mutational status. **Results.** Using AML cell lines with distinct amounts and mutational status of Flt3 we see an increased phosphorylation of CREB upon FL stimulation only in cells with Flt3-wt. These results was verified to be Flt3 specific by using the Flt3-inhibitor PKC412 (Figure 1). Incubation of the cells with PKC412 before FL stimulation totally abolishes the phosphorylation of CREB while the Bcr-Abl specific kinase inhibitor Imatinib had no effects on CREB phosphorylation. As activated receptor tyrosine kinases are known to activate CREB through Erk1/2, we tried to inhibit Erk1/2 using the specific inhibitor U0126. However, we still see CREB activation upon FL stimulation without Erk1/2, but in Flt3-wt cells only. Our data suggest that this FL-mediated activation of CREB occurs via PI3K/Akt in Flt3-wt cells. To verify that activated CREB transcribe mRNA and translates it further into protein we used a CREB promoter assay. After 24 hours we found a significantly increased FL-mediated translation in Flt3-wt cells compared to Flt3-ITD cells. Measured by pPCR, CREB target genes like TNFalpha and SNF1LK was significantly up regulated only in Flt3-wt after 2h FL stimulation. ITDs in Flt3 are known to give a constitutive active receptor, our results suggest that this active signaling response differs from the FL-induced signaling seen in Flt3-wt cells. **Conclusions.** Stimulation of Flt3 with its ligand FL leads to activation of CREB in Flt3-

wt cells independent of Erk1/2. This activation leads to both transcription and translation of Flt3 target genes. However, in Flt3-ITD cells, the constitutive active receptor seems not to transcriptionally activate CREB. These differences need to be further explored in order to evaluate the potential prognostic impact.

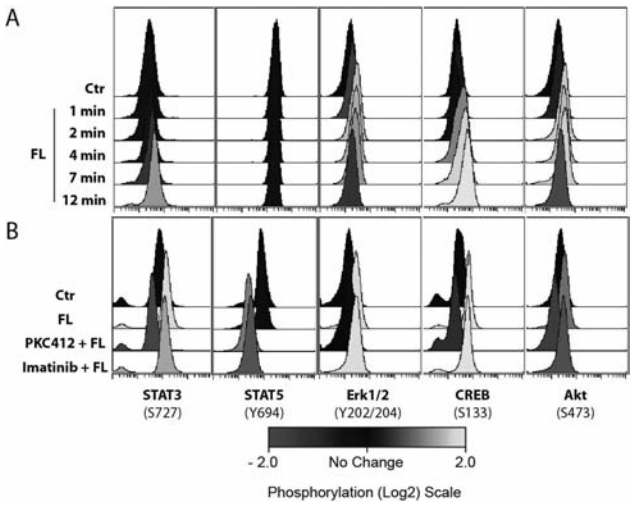

Figure 1. Flt3-Lignad(FL) respond in leukemic EOL-1 cell line. (A) FL stimulation (50 ng/ml). (B) Inhibitors were given 1 hour before FL.

0918

OXIDATIVE STRESS AND C-JUN-AMINO-TERMINAL KINASE ACTIVATION INVOLVED IN APOPTOSIS OF MOLT4 CELLS INDUCED BY DISULFIRAM/COPPER COMPLEX

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Background. Disulfiram (DS), an old drug clinically used for alcoholism, was reported to have antitumor effects and copper (Cu) could significantly enhance the DS-induced cell death. We have recently demonstrated disulfiram/copper (DS/Cu) resensitized doxorubicin resistant leukemia cells to doxorubicin through activation of stress related signaling pathway (c-Jun-amino-terminal kinase, JNK), but the messengers responsible for its activation remain unidentified. In many instances, stress-activated protein kinases (SAPK)/JNK signaling mediates the link between oxidative stress and cell death. **Aims.** To determine the killing effect of DS/Cu complex on human acute lymphoblastic leukemia cells Molt4 and investigate whether oxidative stress and JNK signaling pathway were involved. **Methods.** The cytotoxity of DS/Cu complex on Molt4 cells was detected using MTT assay. Annexin-V/PI and DCFH-DA were employed for apoptosis and intracellular ROS level analysis by flow cytometric analysis. Morphological observation of Molt4 cell stained with Hoechst 33342 was underOLYMPUS fluorescence microscope. The anti-oxidative transcription factor NF-E2-related factor 2 (Nrf2) mRNA and protein expression levels were detected by QT-PCR and western blot respectively. The DS/Cu-induced JNK and p-JNK activation was also examined by western blot. Statistical analysis was carried out with a one-way ANOVA followed by Dunnett's test to assess statistical significance (*$p < 0.05$). **Results.** A serial concentrations of DS in combination with a low concentration (1μM) of Cu^{2+} induced cytotoxicity in Molt4 cells with IC_{50} of 0.435±0.109 μM at 24h. The cell morphology (nuclear condensation and apoptotic body formation) and Annexin-V/PI flow cytometric analysis indicated that DSF/Cu induced Molt4 cell death into apoptosis. With the increasing concentrations of DS (0.125, 0.25, 0.5, 1μM), the apoptotic proportion of Molt4 cells increased from 17.75% to 79.5% at 24h. Nrf2 plays a central role in the protection of cells against oxidative. Results of QT-PCT showed that DS/Cu down-regulation Nrf2 gene expression in a concentration dependent manner, consistently, western blot indicated Nrf2 nuclear translocation was also inhibited. As ROS levels are predominantly regulated by the transcription factor Nrf2, We also detected the ROS levels in Molt4 cells and flow cytometric analysis showed DS/Cu induced ROS generation. ROS-mediated signals have been demonstrated to be strong promoters of JNK activation. Western blot in this study also showed DS/Cu complex induced phosphorylation of JNK expression in Molt4 cells, the total JNK was not changed. To further explored the potential role of oxidative stress in DS/Cu complex-mediated apoptosis, Molt4 cells were exposed to indicated concentration of DS/Cu complex in the presence or absence of an antioxidant N-acetyl-L-cysteine (NAC). Results showed that NAC attenuated DS/Cu complex-induced apoptosis, restore Nrf2 nuclear translocation and block JNK activation. **Conclusions.** DS/Cu complex potentiated cytotoxitiy to Molt4 cells through apoptosis induction. Oxidative damage including Nrf2 inhibition and ROS generation, followed by activation of JNK, play a functional role in DS/Cu mediated antileukemic effects.

0919

THE ACUTE MYELOID LEUKEMIA-DERIVED CELL LINE OCI-AML1 IS A MODEL FOR VEGF-C SIGNAL TRANSDUCTION STUDIES

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Background. Vascular endothelial growth factors (VEGFs) and their receptors (VEGF-Rs) are important regulators of angiogenesis and lymphangiogenesis. VEGF-A triggers the activation of the tyrosine kinases VEGF-R1 (FLT1) and VEGF-R2 (KDR) on blood vessel endothelial cells. VEGF-R3 (FLT4), primarily expressed on lymphendothelial cells, is stimulated by VEGF-C and VEGF-D. VEGF-Rs can also be expressed in solid tumors and on leukemic cells. The interaction of receptors with their ligands mediates survival and can lead to proliferation of the malignant cells. Results of our study of 95 leukemia-lymphoma cell lines show that not only *FLT1* and *KDR*, but also *FLT4* is regulated by DNA methylation. **Aims.** To find models for VEGF-C/FLT4 signalling, we tested the FLT4 protein expression on cell lines with unmethylated *FLT4* promoters. OCI-AML1 was the only FLT4-positive cell line which was cytokine-dependent. Cytokine-dependent cell lines are particularly useful models for signalling studies, as cytokine starvation silences receptors and the receptor-downstream enzymes. Addition of a cytokine then activates its receptor and relevant signalling cascades. Here, we set out to test whether cell line OCI-AML1 can be used as model system for VEGF-C/FLT4 signalling. **Methods.** Cell signalling was tested by Western bloting using antibodies against the phosphorylated and unphosphorylated forms of canonical signal transduction kinase targets. RNAs of unstimulated and VEGF-C-stimulated OCI-AML1 cells were reverse-transcribed. Expression array analysis (Affymetrix Gene Chip HG-U133 Plus 2) was performed to detect genes differentially expressed in unstimulated and VEGF-C-stimulated cells. Expression levels of VEGF-C-regulated genes were reanalysed by quantitative real time PCR. Results were confirmed in experiments studying time course and dose dependence of the VEGF-C effect.**Results.** Of the 95 leukemia-lymphoma cell lines tested, 6 strongly expressed *FLT4* mRNA and protein (BV-173, HEL, MEG-01, MHH-CALL2, OCI-AML1, SUP-B15). Among these six cell lines only OCI-AML1 was cytokine-dependent. Stimulation with VEGF-C (125 ng/ml) induced phosphorylation of ERK1/2 (after 5 min) and induced expression of a set of genes, e.g. *Angiopoietin-1* (1.6x after 4h, 3.2x after 48h), ETV5 (2.5x after 4 h; 2.1x after 48h), and FAM101B (2.3x after 4h, 3.7x after 48h). **Summary and Conclusions.** Bisulfite sequencing and methylation-specific PCR of the *FLT4* promoter performed for 95 leukemia-lymphoma cell lines revealed the inverse correlation between promoter methylation and gene expression that is typical for epigenetically regulated genes. Confirming epigenetic regulation of *FLT4*, DNA demethylating agents induced gene expression in *FLT4* methylated cell lines. Cell line OCI-AML1 was the only FLT4 protein expressing cell line that requires cytokines for cell growth. VEGF-C induced phosphorylation of ERK1/2 in starved OCI-AML1 cells and induced expression of specific genes, *Angiopoiein-1* being the most interesting one. Like VEGF-A and the VEGF-Rs *FLT-1* and *KDR*, *Angiopoietin-1* and its receptor Tie2 are important mediators of angiogenesis. These results suggest that VEGF-C may induce expression of Angiopoietin-1 in tumor cells which, in turn might lead to vascularization of the tumor. These results show that OCI-AML1 is a promising new model for VEGF-C/FLT4 signalling studies.

0920

MOLECULAR INTERACTION OF THE SMALL GTPASE RAC1 WITH THE HEMATOPOIETIC PROTEIN DOCK10. EXPRESSION STUDIES OF THE ZIZIMIN SUBFAMILY OF DOCK PROTEINS.

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Background. Dedicator of cytokinesis (DOCK) proteins are a family of guanosine nucleotide exchange factor (GEF) for small Rho GTPases. The Zizimin subfamily includes DOCK9, DOCK10, and DOCK11. Recently, our group described tissue expression of the alternative first coding exon DOCK10 isoforms, designated DOCK10.1 and DOCK10.2 (Alcaraz-Garcia *et al*. Hum Immunol 2011; 72: 531-537). There is evidence that DOCK9 and DOCK11 are

specific activators of Cdc42. Specificity of DOCK10 for Rho GTPases remains to be elucidated. **Aims.** 1) To identify the isoforms of DOCK9 and DOCK11, and their tissue expression; 2) to study the cytosolic/nuclear distribution of the three Zizimin proteins; and 3) to define the specificity of DOCK10 for Rho GTPases. **Methods.** RT-PCR and qPCR methods were designed to identify the suspected isoforms of DOCK9 and DOCK11. Cytoplasmic and nuclear protein extracts were obtained from primary cells and cell lines and assayed by western blot analysis. GST fusions with "classical"Rho GTPases were used to pulldown protein extracts from 293T transfected with Zizimin proteins, in nucleotide-free (nf) conditions or loaded with GTP or GDP. **Results.** Four DOCK9 isoforms result from the combination of two alternative starts and two alternative ends. The usage of two alternative first exons gives rise to the components designated DOCK9.1 and DOCK9.2. The alternative splicing of exon 33 gives rise to long (L) and short (S) isoforms, i.e., the components designated DOCK9.L and DOCK9.S. Thus, the isoforms were designated DOCK9.1.L, DOCK9.2.L, DOCK9.1.S, and DOCK9.2.S. One single form of DOCK11 was described. Total DOCK9 was expressed in diverse tissues, being the highest levels found in T cells. DOCK11 expression was more restricted to hematopoietic tissues, being expressed with the highest levels also in T cells. DOCK9, in contrast to DOCK10 and DOCK11, was not expressed in B cells. In the majority of tissues, DOCK9.l.L and DOCK9.1.S were the predominant isoforms and were expressed with similar levels. DOCK10 and DOCK11 located in the cytoplasmic fraction of PBMC. Consistent with previous reports, DOCK9 and DOCK11 interacted with nf Cdc42 but not with GDP- or GTP-loaded Cdc42, nor with Rac1 or RhoA, supporting their role as Cdc42-specific GEFs. Both DOCK10 isoforms interacted with nf Rac1but not with GDP- or GTP-loaded Rac1. With less intensity, DOCK10 isoforms interacted with nf Cdc42 but not with GDP or GTP-loaded Cdc42. They did not interact with RhoA. In addition, the DOCK10 isoforms interacted weakly with nf Rho F, Rac2, RhoG, and RhoD, and did not interact with Rac3, RhoJ, or RhoQ. **Conclusions.** The Zizimin subfamily is a group of 3 proteins mainly expressed in lymphoid tissues. Four DOCK9 isoforms have been described, being DOCK9.1.L and DOCK9.1.S the ones expressed with the highest levels in most tissues. A single isoform of DOCK11 was found. The Zizimin proteins located to the cytoplasm in PBMC. In contrast to DOCK9 and DOCK11 that specifically interacted with Cdc42, DOCK10 interacted with more intensity with Rac1, suggesting that DOCK10 could be a specific GEF for

0921

EXPRESSION ANALYSIS OF S100 PROTEINS IN MYELOPROLIFERATIVE NEOPLASM BY MICROARRAY AND PROTEOMIC STUDIES

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The S100 family represents small acidic proteins with common EF-hand calcium-binding motifs. S100 proteins mainly exhibit a diverse cell- and tissue-specific expression pattern with intra- and extracellular functions. Furthermore, several S100 proteins have also been reported to play important roles in cancer progression and suppression, as well as cell proliferation and differentiation. S100 proteins activate some intracellular transduction pathways including JAK/STAT signaling pathway constitutively activated in myeloproliferative neoplasm (MPN) with JAK2V617F mutation. Using microarray analysis, we studied the cancer related S100 genes expression profile of hematopoietic $CD34^+$ progenitor cells and granulocytes from peripheral blood of patients with essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). The proteomic studies of S100 proteins in granulocytes have been performed in 4 groups: PV, ET, and PMF with JAK2 mutations and ET/PMF with no JAK2 mutation. Cancer promoters S100A4 and S100A6 genes expression have statistically significant increases in hematopoietic progenitor cells of ET and PV patients compared to healthy controls, with decreased expression in granulocytes of MPN patients. S100A4 protein expression is decreased in PV patients in contrast to other MPNs regardless of JAK2 mutation. In addition, S100A4 and S100A6 protein expression is increased in PMF patients versus ET/PMF patients without JAK2 mutation and PV patients, respectively. S100A8/A9 expression in cancer cells has also been linked with tumor development and cancer invasion. S100A8 has largely increased gene expression in hematopoietic progenitor cells of MPN patients, while S100A8 protein expression is increased in PV and PMF patients with JAK2 mutation in comparison to MPN patients with no mutation. S100A9 protein expression is increased in ET and PMF, opposed to counterpart with no JAK2 mutation and PV, and more prominent in ET patients. S100A9 has mostly increased gene expression in hematopoietic progenitor cells and granulocytes of MPN patients, significantly reduced only in ET granulocytes. Tumor suppressor and cell growth inhibitor S100A11 gene expression is decreased both in hematopoietic progenitor cells and granulocytes of MPN patients, whereas the protein expression is significantly increased only in PMF patients with JAK2 mutation versus mutation negative MPN patients. In contrary, proinflamatory S100A12 protein expression is increased in mutation negative MPNs compared to MPN patients with JAK2 mutation, where PMF patients demonstrated the most increased protein expression. S100A12 gene expression, absent in controls, is increased in hematopoietic progenitor cells of PV patients, and occasionally present in ET and PMF patients. In conclusion, our results show that s100 gene expression balance is significantly altered in MPN hematopoietic progenitors, together with modification of protein expression in granulocytes. These results propose a novel role for S100 proteins in phenotypic and genotypic characteristics of MPNs.

0922

TRANSCRIPTOME-WIDE MRNA TARGETS OF THE RNA-BINDING PROTEIN MSI2 INVOLVED IN MYELOID MALIGNANCIES

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Background. Musashi homolog 2 (MSI2) is an RNA-binding protein (RBP) with an RNA-binding domain located in its N-terminus. It was first associated with myeloid malignancies when it was shown to form a fusion protein with HOXA9 in chronic myeloid leukemia (CML). Moreover, expression of wild-type MSI2 is highly up-regulated during CML progression. Additionally, the depletion of MSI2 in human myeloid leukemia cell lines leads to decreased proliferation and increased apoptosis. Expression of MSI2 expression predicts unfavorable outcome in AML patients. MSI2 was suggested as a new prognostic marker and a new therapeutical target in AML. **Aims.** Gene expression in eukaryotes is extensively controlled at the post-transcriptional level by RBPs modulating the maturation, stability, transport, editing and translation of the RNA transcripts they are bound to.Neither the mechanism by which the overexpression of MSI2 contributes to leukemogenesis nor the physiological function of this protein are understood. It is therefore crucial to gain insight into its transcriptome-wide mRNA targets.We aim to establish the physiological role of MSI2 to then better understand the impact of its overexpression in myeloid malignancies. **Methods.** PAR-CLIP (photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation) was developed to identify the transcriptome-wide RBP binding sites by incorporation of the photoreactive nucleoside 4-thiouridine into nascent mRNAs of cultured cells. Using next-generation sequencing, this allows mapping of the exact crosslink sites thus shedding light on the RNA-recognition elements (RREs), bound mRNA and ultimately the function of the studied RBP. **Results.** PAR-CLIP was employed for MSI2. The roughly 200 million generated mRNA reads gave rise to around 20000 clusters meeting stringent quality criteria. We found that cytoplasmically localized MSI2 predominantly binds intronic and 3'UTR mRNAs at roughly equal distribution.We have performed an siRNA knockdown of MSI2 and will correlate the data of genes whose expression is altered to those bound by MSI2 as defined by PAR-CLIP paying special attention to such genes previously published to be dysregulated in myeloid malignancies.We have also bioinformatically defined the RRE of MSI2 and are now in the process of validating it biochemically. **Summary and Conclusions.** We have successfully applied PAR-CLIP to the RBP MSI2, which was previously shown to play a role in myeloid malignancies. For the first time, the direct mRNA targets of MSI2 have been elucidated and the RRE was defined. This serves as the starting point to further investigate the physiological role of this RBP to then analyze the impact of its overexpression in myeloid malignancies.MSI2 has already been proposed as a new target for therapy in AML and our data, especially the newly described RRE, provides the starting point for the design of inhibitors such as RNA decoys containing a high affinity ligand for MSI2.

0923

PHOSPHOPROTEOM ANALYSIS OF CHRONIC MYELOID LEUKEMIA CELLS USING PROTEIN ARRAYS

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Backround. There are probably a number of mechanisms of resistance to tyrosine kinase inhibitors. It has been suggested that in many cases over time from chronic myeloid leukemia (CML) diagnosis, the disease may become partially or totally BCR-ABL independent, driven by other proteins, especially kinases. List of those proteins is probably still incomplete and is increasing rapidly. Knowledge of the other players in CML pathogenesis is of high importance as it might improve both CML patients monitoring and therapy. **Aims**. In our current study, we aim to select proteins differentially activated in CML patients with different responses to kinase inhibitor therapies (imatinib, nilotinib, dasatinib). These proteins might serve as novel biomarkers and therapy targets. We look at the changes in CML cell lines and primary patients´ leukocytes treated with tyrosine kinase inhibitors in vitro. As many proteins are activated/deactivated by phosphorylation at different sites, we aim to study phoshoproteom of CML cells. **Methods.** We have applied commercially available protein arrays to see phosphorylation profiles. Antibody array analyses were optimized and data for selected proteins were checked by Western blot analyses.4.ResultsPreliminary data showed differences in activated pathways in samples of patients with different response to therapy and CML aggressiveness. In our study, we found differences in phosphorylation status of different kinases including Src family (Src, Yes) and others (BTK, ZAP-70). Interestingly, we also found differences in proteins which regulate cellular kinases (CSK) or interact with them (GRB2, GRAP) and influence their effects. Differences in expression and/or activation of such regulators might explain different CML aggressiveness in patients with comparable levels of BCR-ABL. Differences were found upon treating CML cell lines and primary patients´ leukocytes with tyrosine kinase in vitro.**Summary and conclusions.** Protein antibody arrays are novel promising tool to provide complex insights into disease pathogenesis. Complex coverage of the proteome by todays´ available protein arrays allow to reveal important pathways in disease pathogenesis and to find novel markers and targets for therapy.Grant support: NT/12392-4 IGA MZ-CR.

Hemoglobinopathy 2

0924

REDUCTION IN LIVER IRON CONCENTRATION IS CONSISTENT ACROSS SUBGROUPS OF NON-TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS TREATED WITH DEFERASIROX: RESULTS FROM THE 1-YEAR THALASSA STUDY

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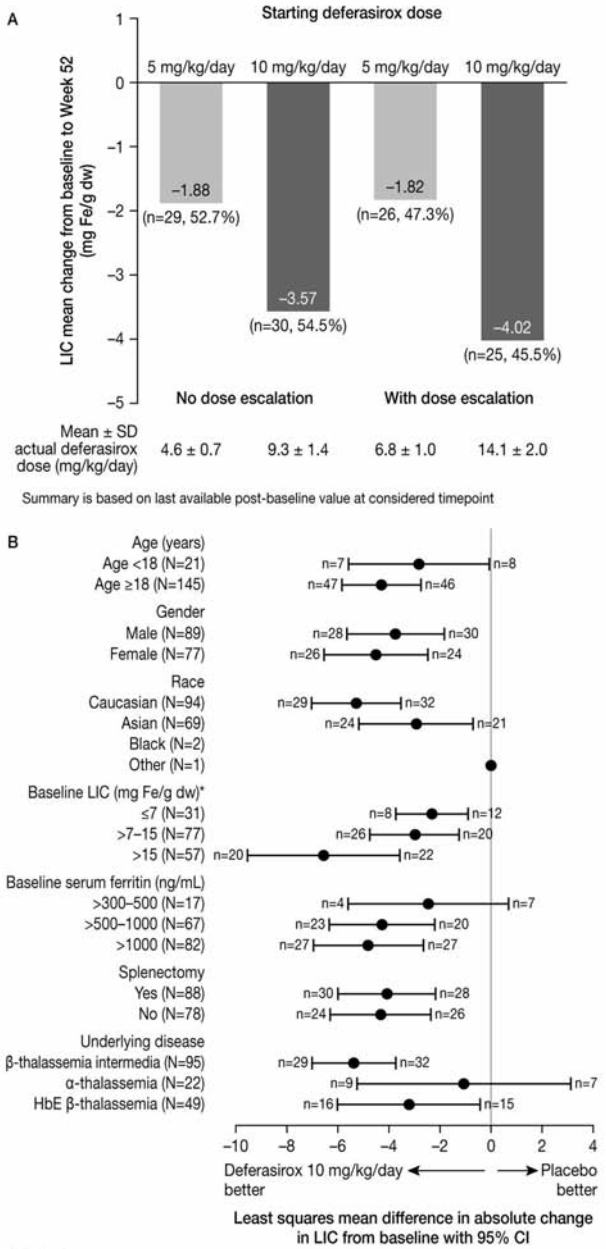
Background. Non-transfusion-dependent thalassemia (NTDT) patients, including β-thalassemia intermedia (β-TI), mild/moderate forms of HbE/β-thalassemia and α-thalassemia (HbH disease) require occasional/no blood transfusions, but are at risk of clinically relevant iron overload as they age. This is mainly due to increased intestinal iron absorption driven by ineffective erythropoiesis, leading to increased risk of vascular, endocrine and bone disease. The 1-year, randomized, double-blind, placebo-controlled THALASSA study assessed efficacy and safety of the iron chelator deferasirox for investigational use in iron-overloaded NTDT patients. **Aims.** To assess the consistency of deferasirox efficacy on liver iron concentration (LIC) in various subgroups of NTDT patients. **Methods.** Study design and inclusion/exclusion criteria for THALASSA have been described previously (Taher *et al. Blood* 2011;118:abst902). Patients (or parents/guardians) provided written, informed consent. Deferasirox dose could be doubled at Week 24 in patients with insufficient response (LIC >7mg Fe/g dw and reduction <15% compared to baseline). Primary efficacy endpoint was the absolute change from baseline in LIC at Week 52 (last-observation-carried-forward); analyzed here by subgroups: age, gender, race, baseline LIC/baseline SF, splenectomy, underlying thalassemia syndrome and requirement for dose increase. Estimates were also obtained from an ANCOVA model with treatment as factor and baseline LIC as covariate. **Results.** 166 patients with β-TI (n=95), HbE/β-thalassemia (n=49) or α-thalassemia (n=22) were randomized to starting deferasirox doses 5 mg/kg/day (n=55)/matching placebo (n=28) or 10 mg/kg/day (n=55)/matching placebo (n=28). At Week 24, dose was doubled in 26 (47.3%) and 25 (45.5%) patients receiving starting deferasirox doses 5 or 10 mg/kg/day, respectively; mean±SD actual deferasirox doses were 5.7±1.4 and 11.5±2.9 mg/kg/day, respectively. At Week 52, LIC significantly decreased from baseline by least-squares mean -1.95 and -3.80mg Fe/g dw in the deferasirox 5 and 10 mg/kg/day groups, and increased by 0.38mg Fe/g dw in the placebo.Patients receiving deferasirox 10 mg/kg/day throughout the study achieved mean LIC reduction of -3.57mg Fe/g dw; those escalated from 10 to 20 mg/kg/day achieved -4.02mg Fe/g dw. LIC reduction for patients receiving deferasirox 5 mg/kg/day with/without escalation to 10 mg/kg/day was similar (-1.82mg Fe/g dw with escalation; -1.88mg Fe/g dw without escalation; Figure A). Mean LIC decreased in both deferasirox dose groups irrespective of analyzed subgroups. In general, the direction of the estimated differences by subgroup was consistent with the primary efficacy analysis (Figure B).No relevant differences were observed in the deferasirox adverse event profile across the analyzed subgroups. Laboratory evaluations (shifts in aspartate aminotransferase, alanine aminotransferase, serum creatinine, creatinine clearance, urinary protein/creatinine ratios, or change in hematology variables from baseline) also did not show relevant differences across subgroups. **Summary and Conclusions.** Based on this subgroup analysis, larger reductions in LIC were achieved in NTDT patients receiving starting deferasirox 10 mg/kg/day, with greatest reductions in those escalated to 20 mg/kg/day. Although not powered to determine differences between subgroups and low patient numbers in several subgroups precluded clinically relevant conclusions, LIC decreased from baseline in all subgroups and there

were no observed differences in safety between subgroups. Thus, deferasirox efficacy and safety is consistent across this diverse group of NTDT patients.

Figure. (A) Changes in LIC by requirement for dose escalation during the study; and (B) least squares mean with 95% confidence interval for differences in absolute change in LIC from baseline to Week 52 between deferasirox 10 mg/kg/day and all placebo combined by subgroups



*N=1 missing;
n is the number of patients for deferasirox 10 mg/kg/day (left) and all placebo combined (right);
dots represent least squares mean differences between treatment groups and lines represent 95% CI

0925

FIBROSIS IN CARDIAC SIDEROSIS: UPDATE ON HISTORICAL PERSPECTIVES

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Background. Heart failure related to cardiac siderosis is a major cause of death in transfusion dependent anemias, and in particular beta-thalassaemia major. Replacement fibrosis been reported as causative of heart failure in siderotic cardiomyopathy in historical reports, but the relevance of these is now controversial as they do not accord with the reversible nature of heart failure in cardiac siderosis achievable with intensive iron chelation. **Aims.** We aimed to assess the prevalence of fibrosis in cardiac siderosis in the modern era. **Methods.**Ten whole hearts (9 beta-thalassaemia major, 1 sideroblastic anaemia) were examined for iron loading and tissue fibrosis. Of these patients, 5 died from heart failure, 4had cardiac transplantation for heart failure, and 1 died from a stroke with no heart failure. Heart samples were quantified for iron content using inductively coupled plasma atomic emission spectroscopy. Fibrosis was quantified by computer using picrosirius red (PSR) staining and expressed as collagen volume fraction (CVF) with normal value <3%. **Results**. There was no significant macroscopic replacement fibrosis in any heart. In only 2 hearts was fibrosis identifiable, and this was predominantly interstitial but with low CVF: in one patient who died from a stroke with no cardiac siderosis, CVF was 5.9%; in the second patient who had heart failure, the CVF was 2%. In the remaining 8 patients, no fibrosis was seen despite all having severe cardiac siderosis and heart failure (mean CVF 1.86% ±0.87%). **Conclusions.** This study shows that in the modern era, cardiac fibrosis is not responsible either for heart failure, or death from heart failure in patients with transfusion dependent anaemia, including beta-thalassaemia major patients. Replacement fibrosis was not seen and the minor interstitial fibrosis identified in 2 patients was not contributory to heart failure. The absence of cardiac fibrosis accords with the reversible heart failure seen in iron overload cardiomyopathy These findings are in marked contrast to the major historical pathological series in transfusion dependent patients by Engle (1964) and Buja (1971). The explanation for the change in prevalence of cardiac fibrosis over 40 years can only be subject to speculation. The likely possibilities include improved blood transfusion preventing damage from excessive cardiac output, and the introduction of the iron chelator deferoxamine which came into widespread clinical use in the 1970's. Our data therefore suggests that modern management prevents cardiac fibrosis, despite cardiac siderosis ultimately being a common cause of cardiac death.

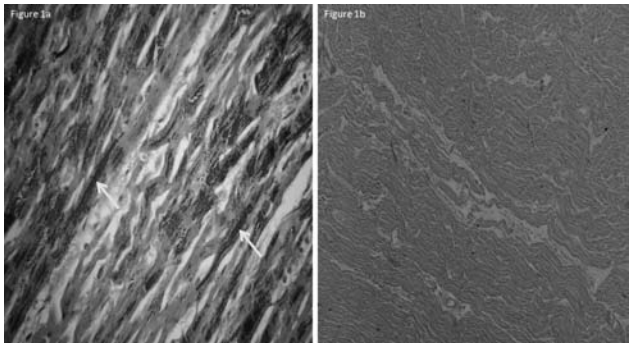

Figure 1a: Heavily iron loaded myocardium (Perl's stain). Nearly all myocytes showed homogenous positive purple/blue iron granules (arrows) within the cytoplasm.b: Picrosirius red staining of myocardium from a patient with severe cardiac siderosis. No significant fibrosis is sen

0926

IRON OVERLOAD LOWERS THE GLOMERULAR FILTRATION RATE ESTIMATED BY CYSTATIN C IN PATIENTS WITH THALASSEMIA MAJOR

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Background. Thalassemia major is an inherited hemoglobinopathy requiring frequent lifelong blood transfusions. Patients suffer from complications related to necessary regular blood transfusions including iron overload. The renal complications have not been well studied. **Aims.** In the current study, we analyzed the impact of iron overload on the glomerular filtration rate (GFR) in children and adults with thalassemia major. **Methods.** One hundred and fifty five adults and children with thalassemia major treated at our center were eligible for the cross sectional study. None had known abnormal renal function. We used Cystatin C to estimate the GFR given the previous reports of improved accuracy and correlation with measured GFR using this method. Univariable and multivariable linear regression modeling was used to study the impact and adjust for the multiple baseline characteristics on the GFR as estimated by serum Cystatin C. These included age, gender, diabetes mellitus and type of chelation therapy.Results. Of the 155 patients approached, 6 patients were excluded (5 declined consent, 1 was pregnant). The mean age was 17.3 years (standard deviation [SD] 9, range 2.5-38.5) with 78 males and 71 females. The mean body mass index was 19.2 (SD 4.7, range 10.6-35.8). The mean systolic and diastolic blood pressures were 106 mmHg (SD 10, range 81-138) and 64 (SD 10, range 42-8) respectively. Eight and 9 patients had non-insulin dependent and insulin dependent diabetes mellitus respectively. Twenty-six patients were on combined chelation therapy (deferiprone and deferoxamine), 58 on deferiprone (mean dose 92mg/kg/day), 62 on deferasirox (mean dose 37mg/kg/day) and 1 on deferoxamine (dose 29mg/kg/day). The mean ferritin level was 1913 ng/ml (SD 1972, range 70-11927). The mean serum creatinine and urea concentrations were 39 micmol/l (SD 14, range 3.6-83) and 5 mmol/l (SD 4, range 1.4-46) respectively. The mean GFR estimated using Cystatin C was 105 ml/min (SD 28, range 44-224). In the univariable analyses, ferritin (coefficient = -0.003; p=0.004), diabetes status (coefficient = 22.8; p<0.001) and chelation type (coefficient = -13.23, p<0.001) were statistically significant. After adjusting for the baseline differences using multivariable modeling, ferritin (coefficient = -0.002; p=0.018) and diabetes status (coefficient = 23.9, p<0.001) remained statistically significant. **Conclusions.** In patients with thalassemia major, iron overload is associated with lower GFR. This is likely a direct effect of iron on glomerular function. Diabetes mellitus status is associated with higher GFR which is likely related to hyperfiltration seen typically in early diabetes. Further studies are needed to confirm these findings.

0927

ESTIMATION OF LIVER IRON CONCENTRATION BY SERUM FERRITIN MEASUREMENT IN NON-TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS: ANALYSIS FROM THE 1-YEAR THALASSA STUDY

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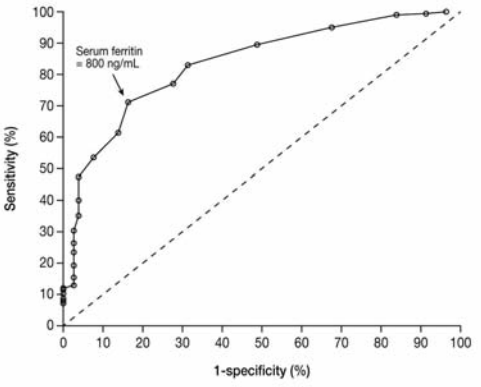
Background. Many non-transfusion-dependent thalassemia (NTDT) patients develop elevated liver iron concentration (LIC) over time, increasing the risk of clinical complications. In NTDT patients, data suggest that serum ferritin (SF) levels underestimate iron load. Although non-invasive magnetic resonance imaging (MRI) assessment to determine LIC and guide chelation therapy is more robust clinically, due to limited access in many parts of the world, it would be practical to also use SF levels to manage iron overload. The randomized, double-blind, placebo-controlled THALASSA pivotal study assessed the efficacy and safety of deferasirox for investigational use in iron-overloaded NTDT patients and provided a large dataset to allow exploration of the relationship between LIC and SF. **Aims.** To evaluate using Receiver Operating Characteristic (ROC) analysis whether SF can be used to estimate LIC and determine SF cut-offs with high positive predictive value (PPV) and low negative predictive value (NPV) in predicting clinically relevant LIC thresholds in NTDT patients. **Methods.** THALASSA enrolled iron-overloaded NTDT patients (LIC ≥5mg Fe/g dw and SF >300ng/mL) aged ≥10 years. After obtaining written, informed consent from patients (or parents/guardians), NTDT patients underwent eligibility screening prior to enrollment. Using data from all screened patients with available SF (measured at a central laboratory) and LIC (measured using validated R2 MRI and read centrally), a ROC analysis was performed using LIC ≥5 and ≥7mg Fe/g dw, the levels used to initiate and dose increase deferasirox therapy, respectively during THALASSA. PPV was calculated as the percentage of true positives (based on LIC) among all positives (based on SF) and NPV was calculated as the percentage of true negatives (based on LIC) among all negatives (based on SF). **Results.** 282 patients with β-thalassemia intermedia, HbE/β-thalassemia or α-thalassemia had LIC and SF determined at screening. Patients with SF >800ng/mL had a high probability (91.7%) of LIC ≥5mg Fe/g dw and SF >2000ng/mL was highly predictive (92.9%) of LIC ≥7mg Fe/g dw. Among patients with SF ≤800ng/mL, 46.4% had LIC ≥5mg Fe/g dw. To assess SF thresholds for potential clinical guidance of chelation therapy, percentages of patients with LIC <3 (iron chelation stopping criterion), <5 and <7mg Fe/g dw at SF cut-off of 100 (used in THALASSA as a SF-based treatment stopping criterion) and 300ng/mL were calculated. Three patients had SF ≤100ng/mL and all had LIC <3mg Fe/g dw. At the SF 300ng/mL cut-off, 12/15 (80.0%) patients had LIC <3mg Fe/g dw, 13/15 (86.7%) had LIC <5mg Fe/g dw, and 15/15 (100%) had LIC <7mg Fe/g dw. **Summary and Conclusions.** Although underlying factors may affect the SF/LIC correlation, this ROC analysis suggests that SF could be useful in some NTDT patients to guide iron chelation. Patients with SF >800ng/mL had a high probability of LIC ≥5mg Fe/g dw, at which level deferasirox was initiated in THALASSA; in patients with SF ≤800ng/mL, LIC confirmation of iron overload is desirable. SF >2000ng/mL was highly predictive of LIC ≥7mg, at which level deferasirox dose increase was allowed in THALASSA. SF ≤300ng/mL appears adequate and safe to interrupt deferasirox therapy.

Figure. ROC curve for using serum ferritin to predict LIC ≥5 mg Fe/g dw based on all screened patients with screening serum ferritin and LIC



0928

PREVALENCE OF CARDIAC IRON OVERLOAD IN PATIENTS WITH TRANSFUSION-DEPENDENT ANEMIAS: DATA FROM THE RANDOMIZED, ACTIVE-CONTROLLED DEFERASIROX CORDELIA TRIAL

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Background. As heart failure related to cardiac iron is a major cause of death in patients with β-thalassemia major and Diamond-Blackfan-anemia (DBA), it is important to understand the prevalence of cardiac siderosis in this population. Cardiac T2*<20ms indicates cardiac iron outside the normal range, with T2*<10ms being associated with highest risk of heart failure and arrhythmia. No studies have had sufficient sample size to examine the distribution of cardiac siderosis across geographic regions. **Aims.** To examine the prevalence and distribution of cardiac iron overload by geographic regions in a large cohort of patients with transfusion-dependent anemias. The relationships between T2* and other iron parameters are also examined. **Methods.** Enrollment is complete for the multicenter, open-label, randomized, Phase II 1year clinical trial evaluating deferasirox versus deferoxamine for the treatment of cardiac siderosis in patients aged ≥10 years with β-thalassemia major, DBA, sideroblastic anemia or Low/Int-1 risk myelodysplastic syndromes (MDS) and history of ≥50 transfusions (CORDELIA; NCT00600938). Data received by Jan 9, 2012 are reported for screened patients. Patient characteristics were summarized by T2* categories and by geographic regions (West: Canada, Cyprus, Italy, Turkey, UK; Middle East: Egypt, UAE, Lebanon; Far East: Taiwan, Thailand, China). Correlations were evaluated using Pearson's correlation coefficient (r). Left ventricular ejection fraction (LVEF) below the lower limit of normal (LLN) was identified using Westwood criteria (males <59%; females <63%). **Results.** 923 patients were screened (99.1% β-thalassemia major, 0.1% DBA, 0.4% MDS 0.3% other; 54.5% male; mean age, 19.2 ± 7.8 years). 83.3% of patients had received >100 transfusions and the majority (98.4%) had received previous chelation therapy. 49.7% of patients had undergone splenectomy.Overall, 20.1% of patients had T2*10-≤20ms; 16.8% had T2*<10ms (West 20.6%, Middle East 12.4%; Far East 20.5%. Furthermore, 64.6% of patients had LIC≥15mg Fe/g dw, and LIC varied between regions (Table).

Table: Prevalence of iron overload in patients with transfusion-dependent anemias, by geographic region.

	Overall (n=923)	Western (n=258)	Middle East (n=462)	Far East (n=203)
T2*	n=755	n=233	n=346	n=176
Geometric mean (95% CI), ms	21.7 (20.7, 22.8)	19.4 (17.8, 21.2)	24.4 (22.8, 26.1)	20.0 (18.0, 22.2)
Cardiac T2* categories, n (%)				
<6ms	41 (5.4)	13 (5.6)	16 (4.6)	12 (6.8)
6–<10ms	86 (11.4)	35 (15.0)	27 (7.8)	24 (13.6)
10–≤20ms	152 (20.1)	59 (25.3)	57 (16.5)	36 (20.5)
>20ms	476 (63.0)	126 (54.1)	246 (71.1)	104 (59.1)
LIC	n=757	n=242	n=326	n=189
Mean (SD), mg Fe/g dw	25.9 (17.0)	19.4 (14.7)	25.4 (16.3)	35.1 (16.9)
LIC categories, n (%)				
≥15 mg Fe/g dw	489 (64.6)	124 (51.2)	210 (64.4)	155 (82.0)
7–<15 mg Fe/g dw	148 (19.6)	53 (21.9)	75 (23.0)	20 (10.6)
<7 mg Fe/g dw	120 (15.9)	65 (26.9)	41 (12.6)	14 (7.4)

Median serum ferritin was 3739ng/mL (West 2272, Middle East 3804, Far East 5261ng/mL).LIC was highest in patients with T2*<6ms (36.0 [15.0]mg Fe/g dw), and was also elevated in patients with T2*>20ms (LIC 23.5 [16.1]mg Fe/g dw). Mean LVEF was 63.8, 63.8, 66.4 and 67.8% in patients with T2*<6, 6-<10, 10-≤20 and >20ms, respectively. Overall, 12.1% of patients had LVEF below LLN. The proportion of patients with LVEF below LLN was higher in low T2* categories (24.4, 22.4, 15.1 and 8.1% in T2*<6, 6-<10, 10-≤20 and >20ms, respectively).There was no clinically useful correlation between T2* and age (r=-0.054), LIC (r=-0.213), serum ferritin (r=-0.250) or LVEF (r=0.179). **Summary/Conclusions.** Data from this large cohort of patients with transfusion-dependent anemias allowed examination of cardiac siderosis by geographical region. Overall, the prevalence of cardiac siderosis was slightly lower but similar to previous reports (T2*10-≤20ms, 20.1% vs 22.2%; T2*<10ms, 16.8% vs. 23.5% [Daar *et al*. 2008]). Lower levels of cardiac siderosis in Middle Eastern patients (also observed by El-Beshlawy *et al*. 2010) may have influenced overall T2* values in screened patients. Differences in cardiac siderosis between regions may reflect transfusion and chelation practices, as well as genetic population differences.

0929

ARE THE PREFERENTIAL PATTERNS OF MYOCARDIAL IRON OVERLOAD PRESERVED AT THE CMR FOLLOW-UP?

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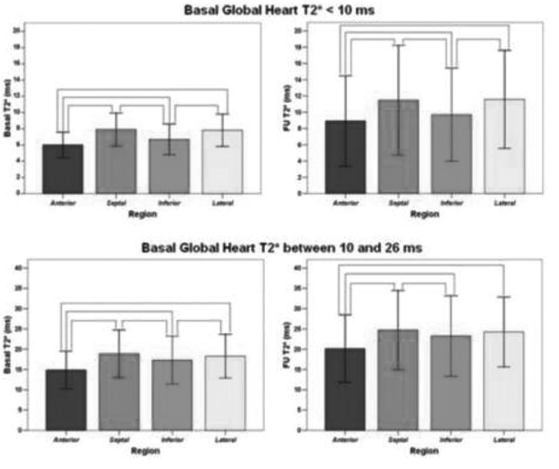
Background. T2* multislice multiecho cardiac magnetic resonance (CMR) allows quantification of the segmental distribution of myocardial iron overload (MIO). **Aims.** This study aimed to determine if a preferential pattern of MIO was preserved between two CMR scans in thalassemia major (TM) patients. **Methods.** Among the 812 TM patients with a CMR follow-up (FU) study at 18±3 months, we selected 259 patients with significant MIO at baseline (global heart T2* <26 ms). Three short-axis views of the left ventricle were acquired and analyzed using a 16-segment standardized model. The T2* value on each segment was calculated, as well as the global value. Four main circumferential regions (anterior, septal, inferior and lateral) were defined.



Results The selected patient population was divided into two groups: severe (N=80, global T2* < 10 ms) and mild-moderate MIO (N=179, global T2* 10-26 ms). During the FU all patients were regularly chelated with excellent/good compliance in the 90 % of the cases. For each group, there was a significant improvement in the global heart as well as in regional T2* values (P<0.0001 for all the pairwise comparisons). For the whole patient population as well as for both two groups, at basal the mean T2* value over the anterior region was significantly lower than the mean T2* values over the other regions and the mean T2* over the inferior region was significantly lower than the T2* values over the septal and the lateral regions. The same pattern was present at the FU, with a little difference for patients with mild-moderate MIO (see figure). **Conclusions**. A preferential pattern of iron store in anterior and inferior regions was present at both basal and FU CMRs, with an increment of T2* values at FU due to a basal CMR-guided chelation therapy. The anterior region seems to be the region in which the iron accumulates first and is removed later. Our data confirm the segmental T2* cardiac MR approach useful for identifying early iron deposit and for tailoring chelation therapy.

0930

IMPROVED IRON CHELATION WITH DEFERASIROX AND ZINC COMBINATIONAL THREAPY IN PATIENTS WITH BETA THALASSEMIA MAJOR: IS IT ADDITIVE OR SYNERGISTIC?

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Background. In this study, we aimed to determine whether administration of zinc had any effect on iron chelation of patients with beta-thalassemia major who were under regular erythrocyte transfusions and chelated with oral deferasirox regularly. **Methods.** Eighty-five cases in a single center who were under regular deferasirox chelation for an average of 3 years (1 to 5) were included.Out of these patients, 46 (45.9%) who have received zinc treatment for at least 1 year and 39 who did not receive zinc treatment were evaluated. The height and weight percentile of the patients and their serum ferritin values have been compared. **Results.** Of the 85 patients with beta thalassemia major who were compliant to medications and follow-up, the mean age was 14.5±7.6 years (3 to 40). 53 patients (62.4%) were male and 32 (37.6%) were female. All of the patients were on deferasirox treatment. The mean dose of deferasirox was 31.15±4.26 (21.4-41.6) mg/kg/day in the all study group. The patients received deferasirox early in the morning. 46 patients (54.1%) used zinc while 39 (45.9%) not. The dose of zinc sulfate was 1x15 mg for 2-10 years of age and 2x15 mg for patients above 10 years of age. The patients received zinc sulfate before going to bed. All of the patients were on zinc treament by the age of 2; however some patients were unable to continue the drug for gastrointestinal problems including abdominal pain and/or vomiting and the drug was ceased by the physician. The deferasirox dose of zinc-users and non-users were mean: 30.82±4.78 and 31.53±3.56 mg/kg/day, respectively. The difference between deferasirox doses among zinc users and non-users were statistically insignificant (p=0.45). The mean duration of deferasirox use was 39.1±10.8 months for zinc users and 36.1±11.6 for non-zinc users, the difference between deferasirox using time was statisticaly insignificant between two groups (p=0.22). At the end of the study, the serum ferritin values of the patients who were zinc-receivers and non-zinc-receivers were found to be 1456±214 and 2019±327 ng/ml, respectively and this value was statistically significantly lower in the patients who received oral zinc supplementation (p=0.01). 26.1% of the zinc-treated patients were 3 percentiles and below in terms of height, while 46.2% of the non-zinc-treated patients are 3 percentiles and below. Though this difference was outstanding, it was statistically insignificant (p=0.054). **Conclusions.** In addition to increasing patient compliance in enhancing iron chelation activity, increasing the dosage in monotherapies or applying combined treatment; supporting the treatment in deferasirox-receiving patients with orally given zinc may decrease the growth delay, and also might enhance the activity of the chelation significantly.

0931

INTERIM RESULTS FROM THE MULTICENTER COMPACT STUDY OF COMPLICATIONS IN PATIENTS WITH SICKLE CELL DISEASE AND UTILIZATION OF IRON CHELATION THERAPY: A RETROSPECTIVE MEDICAL RECORDS REVIEW

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Background. Sickle cell disease (SCD) patients are living longer and face multiple complications. While early detection and treatment may lead to prevention or delayed onset of complications, evidence-based standards for screening and treatment of SCD complications in adults are lacking. A retrospective chart review may help to identify intervention strategies to minimize or prevent SCD complications and understand transfusion and iron overload management in adult SCD patients. **Aims.** Examine the number and type of SCD complications, blood transfusion patterns, iron chelation therapy (ICT) use, and associated resource utilization in SCD patients ≥16. **Methods.** Medical records of SCD patients ≥16 were retrospectively reviewed at two US tertiary care centers. Patient data were collected from their first visit after age 16 (index date) until the earliest indication of a patient's death, loss to follow-up, or last patient record on file prior to the centers' IRB submission date. Patients were classified into one of three cohorts based on lifetime units of blood received and whether or not they received ICT: <15 units of blood and no ICT (minimally transfused, Cohort 1), ≥15 units of blood and no ICT (Cohort 2), and ≥15 units of blood and ICT (Cohort 3). SCD complication rates were expressed as the number of complications per patient per year (PPPY) and compared among cohorts using rate ratios (RRs). **Results.** A full sample size of over 200 patients is expected. Interim results from 73 patient records reviewed between 08/2011 and 01/2012 (Cohort 1: 15, Cohort 2: 20, Cohort 3: 38) are presented. Mean (range) age at index date was similar across cohorts (23 yrs [16-50]). Mean length of observation was 9.8 years. Patients in Cohort 1 received an average of 1 unit of blood PPPY (p<0.05 vs. Cohort 2 and Cohort 3), whereas patients in Cohort 2 and Cohort 3 received an average of 14 and 11 units per year (p=0.717), respectively. Overall, acute pain crisis (62.6%), priapism (6.4%), infection (6.0%), leg ulcers (3.0%), and acute chest syndrome (1.9%) were the most frequently reported SCD complications. The rate (95% CI) of any SCD complications was the highest in Cohort 1 at 1.23 (1.03-1.42) PPPY, followed by 0.99 PPPY (0.85-1.12) in Cohort 2, and 0.45 PPPY (0.39-0.52) in Cohort 3 (Table 1). Patients in Cohort 1 were more likely to experience SCD complications than those receiving more frequent transfusions (RR [95% CI] Cohort 1 vs. (Cohort 2 + Cohort 3): 1.89 [1.56-2.29]). Among patients receiving more regular transfusions, those receiving ICT were less likely to experience SCD complications than those not receiving ICT (RR [95% CI] Cohort 2 vs. Cohort 3: 2.17 [1.78-2.65]). Similar trends in the rates of SCD complications were observed in outpatient, hospital, and emergency room (ER) visits with the greatest contrast observed in the reduction of ER visits among patients who received ICT (RR [95% CI] Cohort 2 vs. Cohort 3: 5.89 [3.70-9.37]). **Conclusion:** Results from this interim analysis show that the rate of SCD complications is lower in patients receiving more regular transfusions and ICT treatment.

RATE AND RATE RATIO OF SCD COMPLICATIONS BY TYPE OF SETTING

	Cohort 1 n = 15	Cohort 2 n = 20	Cohort 3 n = 38	Cohort 2 + Cohort 3 n = 58	Rate Ratio (RR) Cohort 1 vs. Cohort 2 + Cohort 3	Rate Ratio (RR) Cohort 2 vs. Cohort 3
Total SCD Complications						
Mean per patient n SD	18 n 18	11 n 20	5 n 9	7 n 14		
Rate per patient per year [95% CI]	1.23 [1.03 - 1.42]	0.99 [0.85 - 1.12]	0.45 [0.39 - 0.52]	0.65 [0.58 - 0.71]	RR [95% CI]	1.89 [1.56 - 2.29]	2.17 [1.78 - 2.65]
Outpatient						
Mean per patient n SD	4 n 9	2 n 6	1 n 2	2 n 4		
Rate per patient per year [95% CI]	0.45 [0.35 - 0.60]	0.31 [0.24 - 0.39]	0.10 [0.07 - 0.12]	0.18 [0.14 - 0.21]	RR [95% CI]	2.68 [1.94 - 3.69]	3.11 [2.09 - 4.62]
Hospitalization						
Mean per patient n SD	4 n 5	3 n 8	3 n 6	3 n 7		
Rate per patient per year [95% CI]	0.47 [0.34 - 0.59]	0.32 [0.24 - 0.39]	0.29 [0.23 - 0.34]	0.30 [0.25 - 0.34]	RR [95% CI]	1.57 [1.17 - 2.13]	1.11 [0.82 - 1.50]
Emergency Room						
Mean per patient n SD	2 n 3	4 n 9	1 n 2	2 n 6		
Rate per patient per year [95% CI]	0.25 [0.19 - 0.28]	0.34 [0.28 - 0.44]	0.06 [0.04 - 0.09]	0.17 [0.14 - 0.20]	RR [95% CI]	1.68 [1.14 - 2.47]	5.89 [3.70 - 9.37]

Notes:
Cohort 1: Patients who have received < 15 units of blood in their lifetime and have not received ICT treatment in their lifetime.
Cohort 2: Patients who have received ≥ 15 units of blood in their lifetime and have not received ICT treatment in their lifetime.
Cohort 3: Patients who have received ≥ 15 units of blood in their lifetime and have received ICT treatment in their lifetime.

0932

MYOCARDIAL IRON LOAD AND LEFT VENTRICULAR SYSTOLIC FUNCTION IN CURRENTLY TREATED THALASSEMIA MAJOR PATIENTS

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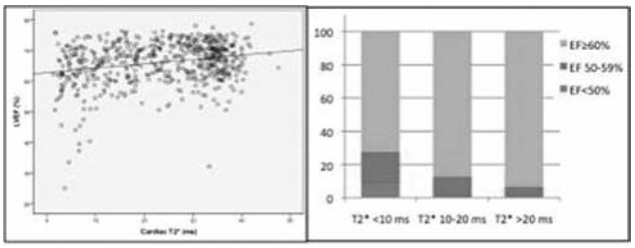
Background. Iron-induced left ventricular (LV) dysfunction is traditionally considered to be the leading cause of heart failure and thus mortality in regularly transfused thalassemia major (TM) patients. Cardiac siderosis can now be accurately quantified by cardiac magnetic resonance (CMR) T2* relaxation time and its functional impact needs to be reassessed in large cohorts of TM patients on modern CMR-guided therapy. **Aims**. To investigate the relationship between myocardial iron load and LV systolic function in a large cohort of consecutive TM patients on modern therapy using CMR T2* imaging. **Methods.** We studied 610 consecutive thalassemia major patients, aged 29±8 years, on regular blood transfusions (pre-transfusion hemoglobin level ≥9.5 g/dL) and standard iron chelation regimens (deferoxamine, deferiprone or both). Patients underwent CMR for the assessment of myocardial T2* and LV ejection fraction (LVEF).
Results. Myocardial T2* was 23±12 ms; 60% of patients had no evidence of cardiac siderosis (T2* ≥20 ms), 21% had mild to moderate iron load (T2* 10-19 ms) and 19% had severe iron load (T2* <10 ms). LVEF was 66±7%; 86% of patients had preserved LVEF (≥60%), 12% had borderline or mildly reduced LVEF (50-59%) and 2% had clearly reduced LVEF (<50%). LVEF was significantly correlated with T2* (Spearman's rho=0.189, p<0.001, Figure, left panel). The prevalence of reduced LVEF differed significantly according to cardiac iron (30% in patients with severe siderosis, 17% in those with mild to moderate siderosis) and interestingly was present even in the absence of cardiac

siderosis (8%, p among the 3 groups <0.001, Figure, right panel). **Conclusions.** The majority of TM patients on modern CMR-guided therapy have no evidence of myocardial siderosis. Systolic LV function is still crucially dependent upon cardiac iron, but LVEF may also be reduced in the absence of cardiac siderosis.



0933

DIABETES MELLITUS AND CARDIAC COMPLICATIONS IN THALASSEMIA MAJOR PATIENTS

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Background. The relationship between diabetes mellitus (DM) and cardiac complications has never been systematically studied in thalassemia major (TM).
Aims. The aim of this study was to evaluate in a large retrospective historical cohort of TM in the cardiovascular magnetic resonance (CMR) era if DM was associated with an higher risk of heart complications. **Methods.** We compared 86 TM patients affected by DM with 709 TM patients without DM consecutively included in the Myocardial Iron Overload in Thalassemia (MIOT) data base where the clinical history is recorded from the birth to the first CMR (years 2006-2010). Myocardial iron overload (MIO) was evaluated by T2* multislice technique. Biventricular function parameters were quantitatively evaluated by cine images. Myocardial fibrosis was evaluated by late gadolinium enhacement CMR technique. All considered cardiac events were developed after DM diagnosis.

Table 1. Logistic regression analysis.

	Adjusted for no-MIO		Adjusted for no-MIO and covariates	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Cardiac complications	4.2 (2.7-6.8)	<0.0001	2.8 (1.7-4.67) #	<0.0001
Heart failure	3.1 (1.9-5.3)	<0.0001	2.3 (1.3-4.1) #	0.003
Hyperkinetic arrhythmias	4.1 (2.2-7.7)	<0.0001	2.2 (1.1-4.4) #	0.023
Myocardial fibrosis	2.1 (1.2-3.6)	0.006	1.9 (1.1-3.3) §	0.021
Heart dysfunction (LV and/or RV)	1.5 (0.4-2.3)	0.093		
Biventricular dysfunction	1.5 (0.8-2.7)	0.235		
LV dysfunction	0.8 (0.4-1.6)	0.487	1.6 (0.4-5.9) *	0.470
RV dysfunction	1.8 (1.0-3.3)	0.048	1.3 (0.7-2.5 *	0.366

Covariates: * age; § endocrine co-morbidity; # age and endocrine co-morbidity

Results. DM patients were significantly older than no-DM patients (37.4 ± 6.2 years vs 32.0 ± 6.7 years, P<0.0001) and showed an higher frequency of endocrine co-morbidity (hypogonadism, hypothyroidism and hypoparathyroidism) (76.7% vs 49.2%, P<0.0001). In DM patients versus no-DM patients we found a significantly higher frequency of cardiac complications (46.5% vs 16.9%, P<0.0001), heart failure (HF) (30.2% vs 11.7%, P<0.0001), hyperkinetic arrhythmias (18.6% vs 5.5%, P<0.0001), and myocardial fibrosis (29.9% vs 18.4%, P=0.008).Table 1 shows odds ratios (OR) and 95% confidence intervals (CI) estimating the relationship between DM and cardiac involvement. Patients with DM had a significant higher risk of cardiac complications, HF, hyperkinetic arrhythmias and myocardial fibrosis, also adjusting for the absence of MIO assessed by T2* CMR (all cardiac segments with T2* ≥ 20 ms) and for the covariates significantly different between groups and significantly associated to the dependent variable. **Summary.** DM increases the risk for cardiac complications, HF, hyperkinetic arrhythmias and myocardial fibrosis irrespective of MIO.

0934

CARDIAC MAGNETIC RESONANCE VERSUS ECHOCARDIOGRAPHY FOR THE ASSESSMENT OF CARDIAC VOLUMES AND FUNCTION IN THALASSEMIA MAJOR PATIENTS

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Background. Cardiac Magnetic Resonance (CMR) and Echocardiography (US) are applied in parallel to thalassemia major (TM) patients for cardiac evaluation and ongoing monitoring. A comparison study between the two techniques involving 135 TM patients demonstrated a mild discrepancy of values in the net volume (diastolic and systolic) but not significantly different ejection fractions (EF). **Aims**. The aim of this study was to compare the agreement of left ventricular (LV) volumes and ejection fraction (EF) by CMR and US in a larger cohort of TM patients.

	LV EDVI (ml/m²)	LV ESVI (ml/m²)	LV SVI (ml/m²)	LV EF (%)
Values				
CMR	86.5 ± 19.9	34.3 ± 11.5	52.2 ± 10.8	60.8 ± 6.7
	(84.2 ÷ 88.7)	(33.0 ÷ 35.6)	(50.9 ÷ 53.4)	(60.0 ÷ 61.5)
US	69.5 ± 17.4	25.3 ± 9.6	44.2 ± 12.0	64.1 ± 6.6
	(67.5 ÷ 71.5)	(24.3 ÷ 26.4)	(42.8 ÷ 45.5	(63.3 ÷ 64.8)
CMR - US				
Difference, mean ± SD	16.9 ± 18.4	8.9 ± 10.9	8.0 ± 12.6	-3.3 ± 7.6
P	<0.0001	<0.0001	<0.0001	<0.0001
Correlation, r	0.536	0.498	0.381	0.279
(P-value)	(P<0.0001)	(P<0.0001)	(P<0.0001)	(P<0.0001)
ICC	0.537	0.519	0.483	0.473
BA limits	-19.1 to 53.1	-12.3 to 30.2	-16.6 to 32.7	-18.2 to 11.6
BA range	72.2	42.5	49.3	29.8

EDVI= end diastolic volume index; ESVI= end systolic volume index; SVI= stroke volume index; EF= ejection fraction

Methods. 307 TM patients (145 M and 162 F; mean age 31.8 ± 8.5 yrs) patients were studied by both echo and CMR (1.5T) within 3 months of each other. All TM patients were enrolled within the MIOT (Myocardial Iron Overload in Thalassemia) network where CMR exams were performed in 8 sites using homogeneous and validated procedures. Steady-state free procession cine images were acquired during 8-second breath-holds in sequential 8-mm short-axis slices from the atrio-ventricular ring to the apex to assess biventricular function parameters quantitatively in a standard way, using MASS® software. Previously demonstrated inter-center variability for the quantification of cardiac function was 6.3%. Echocardiographic studies were carried out in 10 echo labs linked to the thalassemia centers. LV volumes and LVEF were measured by two-dimensional echocardiography using the biplane Simpson's formula. Paired-samples t-test or Wilcoxon test, correlation coefficient, intraclass correlation (ICC), and Bland & Altman plot were used to compare CMR and US parameters. The Cohen's kappa coefficient (k) was used to evaluate the agreement between CMR and echo in identifying borderline EF, mild EF reduction and abnormal EF. **Results**. Table 1 shows the CMR and US parameters, quoted as mean ± SD with 95% CI in round brackets, and the results of the compari-

son between the two techniques. All US volumes were significantly underestimated, especially the end-diastolic volume index, while the US EF was significantly higher than the CMR EF. The correlation between US and CMR volume indexes and EF was significant, but with a low coefficient; especially for the EF. The ICC was good for all the parameters, but never excellent. The Bland Altman plot ranges were wide, with the widest value found for the end-diastolic volume index. In all EF categorizations, k was <0.2, indicating slight agreement. **Conclusions**. In a large CMR versus echocardiographic comparative study in TM patients, metrics of LV volume index and function showed significant systematic inter modality differences. Conversely to the previous findings, CMR and US resulted not interchangeable for LV EF assessment. Of clinical relevance, this study suggests that serial measurements of volumes and function in TM should be performed using the same method and if it is available the more reproducible CMR technique.

0935

THE USE OF APPROPRIATE CALIBRATION CURVES CAN CORRECT THE SYSTEMATIC DIFFERENCES BETWEEN SOFTWARES IN HEPATIC R2* ESTIMATION

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Background. Liver R2* can be used as a surrogate for liver iron concentration (LIC) in iron overloaded subjects. Two different signal decay models, truncated exponential and exponential plus constant, have been validated for R2* estimation and calibrated to liver biopsy. However, reported calibration curves for these two analysis methods differ by 15%. **Aims.** Our aim was to evaluate if the different fitting models yielded significantly different R2* estimates and if these differences disappeared once R2* estimates were converted to LIC units using method-appropriate calibration curves. **Methods.** A single-center (N=45) and a multi-center cohort (N=47) of patients were used. Gradient echo images optimized for R2* estimation were collected at each site according to local clinical practice. R2* values were generated using the CMRTools (truncated exponential model) and custom Matlab code (exponential plus constant model). R2* values were converted to dry weight liver iron concentration using calibrations published by Garbowski and Wood, respectively. Bland Altman analysis was performed with respect to both R2* and LIC estimates. **Results.** Figure 1a shows the Bland-Altman plot of R2* values for the single-center cohort. Results were unbiased for R2* < 300 Hz, but R2* values obtained using exponential plus constant were systematically larger at higher R2* and the difference increased with increasing values. The mean difference was 54.7 ± 85.7 Hz (95% confidence intervals of the difference: lower 28.9 and upper: 80.5 Hz), corresponding to a percentage difference in R2* values of 9.1 ± 11.8%. The bias was eliminated following conversion to LIC units (Figure 1b). The mean difference was -0.8 ± 1.5 mg/g dry (95% confidence intervals of the mean difference: lower -1.3 and upper: -0.3 mg/g dry).Similar differences in R2* estimation were found in the multi-center cohort and the conversion of R2* values to LIC units again removed the disparity.

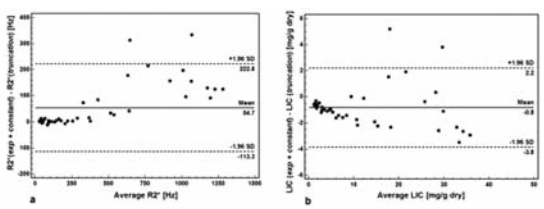

Figure 1. Bland-Altman plot of R2* values (a) and LIC values (b) for the singe-center cohort.

Conclusions. R2* values varied with post-processing method but yielded statistically identical LIC values when technique-appropriate calibration curves were used. LIC, rather than R2* values, should be reported and compared across studies.

0936

LIFETIME COST-UTILITY ANALYSES OF DEFERASIROX IN BETA THALASSAEMIA PATIENTS WITH CHRONIC IRON OVERLOAD: A UK PERSPECTIVE

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Background. Regular blood transfusions for beta thalassaemic patients lead to the accumulation of iron deposits in the body. In order to remove such deposits, iron chelation therapy (ICT) is required. Desferrioxamine (DFO) is administered subcutaneously and has been the gold standard ICT for over 40 years. Deferasirox (DFX) is a newer ICT that is taken orally once daily.**Aims.** In this study, a cost-effectiveness model was developed to estimate the long-term costs and quality-adjusted life-years (QALYs) associated with DFO and DFX treatment in a cohort of beta thalassaemic patients from a UK health service perspective. **Methods.** A 50-year annual cycle state transition cohort model comprised three core health states: alive without cardiac complications, alive with cardiac complications and dead. Within the defined states, patients may experience one or more of the following chronic complications of iron overload: diabetes, hypogonadism, hypoparathyroidism and hypothyroidism. Costs associated with iron chelation and treatment of the listed complications were summed over patients' lifetimes, and utility weights applied to the different health states to inform the estimation of QALYs. The main assumption was that each ICT was equally effective at lowering iron given equal levels of compliance to specified treatment regimens. The model was calibrated to identify sets of convergent input parameter values that best predicted observed data reporting overall survival by lifetime ICT compliance.**Results.** Average lifetime treatment costs for patients receiving DFO were £70,000 higher than DFX: DFX drug costs were £100,000 higher, but DFO equipment costs resulted in overall administration costs that were £170,000 higher. Higher compliance associated with oral DFX administration led to fewer complications. Combined with the quality-of-life effects of an oral mode of administration, an average gain of 4.85 QALYs for DFX was estimated. Thus, in the reference case, DFX dominates DFO, ie, DFX costs less and patients gain more QALYs. The key parameter is the proportion of DFO patients using balloon infusers (the more expensive method - annual cost £13,000 vs £1,400 for pump-based infusion). Sensitivity analyses showed that even when the proportion of patients using balloon infusers is decreased from 79% to 25%, the incremental cost per QALY gained remains well under £20,000. **Summary and Conclusions.** Higher drug costs for DFX are offset by the avoidance of infusion-related equipment costs. Combined with health benefits derived from an oral mode of administration and improved compliance, DFX has a high probability of being an extremely cost-effective intervention compared to DFO.

0937

QUALITY OF LIFE OF CHILDREN WITH SICKLE CELL ANAEMIA: ASSESSING SELF-, PARENT AND HEALTHCARE PROFESSIONAL REPORTS

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Background. Sickle Cell Anaemia (SCA) would be expected to adversely affect the Quality of Life (QoL) of children. There has been extensive research in the QoL of children with SCA; however, the majority of research uses the Paediatric Quality of Life Questionnaire (PedsQL). There have been some inconsistencies with prevalent paediatric QoL measures such as the PedsQL. Furthermore, there have also been reservations with regards to the theory, method and development of current paediatric QoL psychological measures. Therefore, innovative discrepancy measures such as the Generic Children's Quality of Life Measure (GCQ) should be validated using multiple informants such as children with SCA and also adult proxy-reports. Adult proxy-reports may be used when some groups of children with SCA are unable to provide useful QoL self-reports because of age, physically illness, emotionally distressed or too fatigued. **Aims.** The primary objective was to determine whether there was a relationship between the QoL of paediatric patients measured by children with SCA, their parents or carers and healthcare professionals. Secondary objectives were to examine the extent to which demographic indicators and measures of symptom severity predicted child self-reported, parent proxy-reported and healthcare proxy-reported QoL. Moreover, to ascertain whether there was a difference between the QoL of children with SCA compared to their healthy peers. **Methods.** The study adopted an inter-dependent groups, correlational design using a cross-sectional questionnaire, the GCQ. There was a convenience sample of three groups of participants attending a London hospital; chil-

dren with SCA (n = 74) aged between six to sixteen years old (M = 10.61, SD = 3.10), their parents or carers (n = 74) and four members of the healthcare team (n = 74). The three independent groups completed the GCQ as if they were the child. **Results.** Parents or carers and healthcare professionals were able to gauge the QoL of children with SCA. Additionally, child self-reports were not affected by demographic indicators or measures of symptom severity. However, in multivariate analyses, parents or carers who were single reported a lower proxy QoL compared to those who were married or in a relationship, and high parent proxy-reports were also associated with some high stroke risk measurements (Transcranial Doppler examinations) in univariate analyses. Also, higher healthcare proxy-reported QoL were related to fewer days missed from school due to SCA, as well as to less number of crises in univariate analyses. Finally, a comparison between children with SCA and the general child population showed no difference in QoL. **Conclusions.** Adult proxy-reports are valid and may be utilised when children with SCA are unable to provide valuable QoL self-reports. Moreover, parents or carers and healthcare professionals often choose their child's treatment and care in paediatric SCA populations. Their choices are based on an accurate assessment of child's needs. Further research should examine other variables that may affect the QoL of children with SCA such as psychological factors. However, it seems as though children with SCA have adapted to their condition. Moreover, contrary to previous evidence, it appears as though SCA does not impair their QoL.

Table 1. Measures of symptom severity included the total number of crises, days missed from school due to SCA, A&E admissions, or attendance (via parent report and hospital records) and stroke risk measure (TCD).

	Child-self reported QoL	Parent proxy-reported QoL	Healthcare proxy-reported QoL
Total number of crises	-.19	.14	-.36**
Days missed from school due to SCA	-.16	.06	-.40**
A&E admissions, or attendance (parent report)	-.17	.07	-.24
A&E admissions, or attendance (hospital records)	-.14	-.15	-.09
Right ICA	.07	.13	-.04
Left ICA	.07	.26*	-.03
Right MCA	.01	.19	-.17
Left MCA	.13	.29*	-.04

0938

HAEMOGLOBINOPATHY PATIENT ACCESS TO MODERN COMMUNICATION TECHNOLOGY: THE FEASIBILITY OF ALTERNATIVE COMMUNICATION STRATEGIES TO MANAGE CLINIC APPOINTMENTS

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Background. Attendance at our Haemoglobinopathy outpatient clinics often falls to less than 70%, leading to inefficient use of staff time and resources and hindering optimal care. It reduces hospital income, making services less financially viable. Some patients misplace their hospital correspondence or forget appointments because of the 6-12 months between routine visits. **Aims.** In an attempt to improve attendance rates, thereby optimising the use of hospital resources and improving patient care, we explored patient access to electronic modes of communication. **Methods.** We collected data from 51 patients attending Adult Haemoglobinopathy clinics at Central Middlesex Hospital (London,England) between August 2011 and January 2012 (approximately 20% of our patient cohort). Patients were asked about mobile phone ownership, phone access to the Internet, access to a personal computer (P.C), and whether the P.C had Internet access. **Results.** Of these 51 patients, 73% had Sickle Cell Disease, with the remaining 27% having Thalassaemia.28/51 patients (54.9%) were male. The mean age was 39 years, the highest age being 72 years and the lowest being 18 years. 28 patients (54.9%) were in the 31-50 age group.All 51 patients owned mobile phones and 39 (76.5%) had Internet access on their phone. 47/51 patients (92.1%) had access to a P.C and 45/51 (88.2%) had Internet access on these computers.48/51 patients (94.1%) were able to access the Internet in some form. Of those patients who had Internet access on their phones, 87.2% had smartphones with applications. **Summary and Conclusions.** This survey illustrates:1. Ubiquitous access to mobile phones. Hospital communication by phone calls or text messaging would reach almost everyone.2. High levels of access to the Internet (94.1%), allowing communication options such as reminder emails or a dedicated website for patients to manage appointments. The hospital could offer vacated appointments to others, leading to improved use of resources.76.5% had phone Internet, allowing convenient checking of emails (perhaps in preference to using the P.C). A minority of patients (5.9%) had no Internet access at all, meaning our current method of clinic notification (sending letters) may still be required.3. The high level of smartphone ownership. Phone applications ("apps") are another potential form of communication. However, such an app would need to work across different platforms (with cost implications) given that patients own phones from several manufacturers.Using technology such as smartphones and the Internet could be an efficient alternative to printed letters to manage clinic appointments. This survey has demonstrated the widespread, but not universal, access to such technologies within our patient group. Using a range of communication channels may be the best strategy. Possible benefits include improved resource management, optimisation of patient care, empowering patients to manage their healthcare and reducing the use of paper; in accordance with the QIPP (Quality, Innovation, Productivity and Prevention) agenda and their vision of a "paperlight"health service.(QIPP is a UK National Health Service initiative seeking to improve quality of care whilst making efficiency savings to be invested in frontline care).

Red cell biology

0939

CHARACTERIZATION OF THE SEC23B PROMOTER: IMPLICATIONS FOR THE DIAGNOSIS OF CDA II

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Background. *SEC23B* gene is distributed on 53.86 kb of the chromosome 20p11.23. Multiple alternatively spliced transcript variants encoding the same protein (767 aa) have been found for this gene. The SEC23B encoded protein has similarity (66.4%) to yeast Sec23p component of the coat protein complex (COP)II, involved in the anterograde protein trafficking from the endoplasmic reticulum to the Golgi apparatus. Mutations in the *SEC23B* gene cause the vast majority of the cases of Congenital Dyserythropoietic Anemia type II (CDA II), an autosomal recessive disorder characterized by ineffective erythropoiesis, with a reticulocytosis not corresponding to the degree of anemia. To date, more than 50 different causative mutations have been described, localized along the entire coding sequence of the gene (Iolascon, 2011; Russo, 2011; Punzo, 2011). The vast majority of patients had two mutations (in the homozygous or compound heterozygous state), in accordance with the pattern of autosomal recessive inheritance. However, in our cohort approximately the 10% of cases showed only one coding/splicing mutation in heterozygous state. So, we postulate the occurrence of another allelic mutation in the regulatory regions of the gene. **Aims.** To identify and characterize the minimal promoter region of *SEC23B* gene. **Methods.** In order to identify the minimal promoter region of *SEC23B* gene, we established deletion mutants: a 3500 bp upstream region of the gene has been divided into 10 overlapping fragments, amplified from genomic DNA. Each deletion mutant construct has been cloned upstream the luciferase gene into PGL3 vector in the HindIII and XhoI sites. A prediction analysis of transcription factor binding site (TFBS) has been performed by bioinformatic tool MatInspector, from Genomatix web server. Thirteen CDA II independent cases from the International Registry were enrolled for the mutational analysis of the *SEC23B* promoter.Results. By luciferase assay of the deletion mutants we identified the minimal promoter region of the *SEC23B* gene. *In silico* analysis of *SEC23B* upstream region showed the presence of putative binding sites of the main transcription factors involved in the erythroid differentiation, such as GATA1 and KLF1. Finally, we performed a sequencing analysis of *SEC23B* regulatory regions in 13 CDA II independent cases from the International Registry. We found only one patient with an heterozygous mutation in 5'UTR of the gene, which could impair a potential TFBS.Summary/conclusions. Promoter analysis is an essential step on the way to identify regulatory networks. Here, we characterized the minimal active transcriptional region of the *SEC23B* gene. We also predicted the putative GATA1 and KLF1 binding sites: both are involved in peculiar type of inherited dyserythropoietic anemias. The effective binding of these TFBS to *SEC23B* promoter region will be proven by chromatin immuno-precipitation (ChIP) assay, in erythroid cellular models induced to erythroid differentiation. The identification of TFBS could allow the definitive diagnosis of CDA II patients with peculiar clinical phenotypes or those with incomplete pattern of mutations in *SEC23B* coding region.

0940

GLUCOCORTICOID RECEPTOR SELECTIVELY MEDIATES THE EXPANSION OF ERYTHROID PROGENITOR CELLS DURING CHRONIC RESTRAINT STRESS

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Background. The stress-induced erythropoiesis is critical for survival and recovery during various pathophysiological conditions, and its inappropriate activation may predispose to leukemic transformation. The spleen provides a special microenvironment favorable for rapid expansion of erythroid progenitors in response to stressful stimuli. Stress erythropoiesis has been studied primarily in murine models of anemia, but the erythropoietic effects of chronic restraint, that is both physical and psychological stressor, remain largely unknown. Furthermore, the target cell and the physiological effects of glucocorticoids in stress-induced erythropoiesis have been elusive. **Aims.** The purpose of the present study was to investigate the effects of chronic restraint stress on committed erythroid progenitor cells in mouse spleen and to examine the role of glucocorticoid receptor (GR) in restraint-induced changes. **Methods.** Adult male CBA mice were subjected to 2 h daily restraint stress for 7 or 14 consecutive days, and changes within erythroid progenitor cells were analysed in the spleen. The role of GR was assessed by pretreatment of mice with GR antagonist mifepristone (RU486) dissolved in DMSO. To this end, the mice were randomly assigned to five weight-matched groups: 1) R-restraint group exposed to 2 h daily restraint stress during 7 consecutive days; 2) RU486+R group, treated with mifepristone (50 mg/kg) 60 min prior to daily restraint; 3) RU486 group, received only the daily dose of mifepristone; 4) DMSO+R, vehicle injected restraint group; and 5) control group, simply handled daily. *Results.* Chronic exposure to 2 h daily restraint stress resulted in markedly increased number of erythroid progenitors in mouse spleen. Compared to untreated control, there was a significant increase in both early and late committed erythroid progenitors, the erythroid burst (BFU-E) and colony forming (CFU-E) units, after 7 and 14 days of restraint stress. In addition, plasma erythropoietin (Epo) levels were significantly elevated in chronically stressed animals. Pretreatment with RU486 completely abolished the stimulatory effect of chronic restraint stress on CFU-E formation in mouse spleen. In contrast, blockade of GR had no influence on restraint-induced stimulation of BFU-E growth. **Conclusions.** These findings indicate that GR is required for increased expansion of splenic Epo-dependent CFU-E progenitors during chronic restraint stress, suggesting the involvement of GR/Epo-receptor crosstalk mechanism in stress-induced erythropoietic response.

0941

HEREDITARY SPHEROCYTOSIS WITH NORMAL RED CELL SPECTRIN AND REDUCED EMA BINDING IS PREDOMINANTLY CAUSED BY MUTATIONS IN BAND 3

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Background. Hereditary spherocytosis (HS), one of the major red blood cell (RBC) membrane disorders, has heterogenic molecular causes but is in general characterized by low binding of eosino-5'-maleimide (EMA) to the outer surface and decreased osmotic resistance (OR) of the cells. Because RBC spectrin, a main cytoskeletal protein, is often decreased as a primary or secondary cause of disease, we investigated its presence in relation to EMA binding.**Aims.** EMA binds predominantly to band 3 and because all HS patients show low EMA binding, we were interested in the incidence of band 3 mutations being the primary cause of HS in subgroups of patients with and without spectrin deficiency. **Methods.** We examined 677 patients suspected for RBC membrane disorders by RBC morphology, osmotic resistance, EMA binding and spectrin content. Diagnosis of hereditairy spherocytosis (HS) was based on low EMA binding accompanied by the presence of spherocytes and/or low OR. Two groups of HS patients, with and without spectrin deficiency, were screened for mutations in *SLC4A1*, the gene encoding band 3, and for band 3 content.*Results.* A majority of HS patients showed low spectrin levels and reduced EMA binding. Quantification of RBC spectrin, binding of eosino-5'-maleimide (EMA) to the RBC surface, and measurement of the OR revealed a subgroup (36%) of spherocytosis patients with low EMA-binding but without spectrin deficiency. In this subgroup, HS was caused mainly (60%) by mutations in *SLC4A1*. Twenty-four different mutations associated with low levels of band 3 protein were detected, the majority of which were novel. In HS patients without spectrin deficiency the band 3 levels were not significantly decreased, and only 11% carried a mutation in *SLC4A1.* Detected mutations were correlated to band 3 protein levels by Western blotting. The overall incidence for *SLC4A1* mutations in patients with HS is 30%.**Conclusions.** Our results reveal the discriminative value of measuring both spectrin content and EMA binding for the identification of HS due to aberrant band 3. This enables a subdivision of HS. We conclude that aberrant band 3 can cause spherocytosis without affecting red cell spectrin levels, whereas the decreased EMA binding seen in HS patients with decreased spectrin content is likely caused by cytoskeletal rearrangements.

0942

CONSEQUENCES OF DMT1 MUTATION ON ERYTHROPOIESIS

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Background. Deficiency of the divalent metal transporter 1 (DMT1) leads to the development of hypochromic microcytic anemia associated with ineffective erythropoiesis. In contrast to *mk/mk* mice (strain MK/ReJ), a mouse model with DMT1 mutation, majority of DMT1-mutant patients presented with hypersideremia and hepatic iron overload. **Aims.** The aim of this study was to characterize erythropoiesis in *mk/mk* mice propagated on novel 129S6/SvEvTac background. (Gunshin et al, J Clin Invest 2005;115(5):1258-66; provided by Dr. Fleming, Boston). **Methods.** The iron status parameters and tissue non-heme iron content in 129S6/SvEvTac- *mk/mk* mice were evaluated. Colony forming assays were used to analyze the *in vitro* growth of DMT1- mutant erythroid progenitors. Differentiation of erythroid precursors was assessed by flow cytometry analysis. The extent of apoptosis of erythroid precursors and erythrocytes was determined by analysis of Annexin V binding. The expression of key factors of the bone marrow-hepcidin axis was evaluated.Results. The 129S6/SvEvTac-*mk/mk* mice showed significantly increased plasma iron levels when compared to wild-type littermates (48.6±11.7 μM/L *vs.* 33.7±3.7 μM/L) but reduced non-heme iron content in the spleen (49.9±11.9 μg/g *vs.* 535.8±125.4 μg/g) and liver (14.2±3.3 μg/g *vs.* 67.3±18.6 μg/g). Colony forming assay revealed abnormal morphology of *mk/mk* erythroid colonies, reduced number of *mk/mk* CFU-E (164±25 *vs.* 283±50) and BFU-E (9±4 *vs*. 22±5) colonies, and twofold reduction in cellularity of *mk/mk* BFU-Es in comparison to colonies of wild-type mice. Flow cytometry analysis of viable Ter119/CD71 double-stained cells showed that immature erythroblasts predominate in the bone marrow and spleen of *mk/mk* mice when compared to wild-type mice; the difference is more profound in the spleen, reflecting the dramatic expansion of splenic erythropoiesis in *mk/mk* mice. The *in vivo* apoptotic rate of Ter119+ erythroblasts in the bone marrow and spleen was 4.4-fold and 6.6-fold higher in *mk/mk* mice than in the wild-type littermates. A markedly increased susceptibility of *mk/mk* erythrocytes to undergo apoptosis after exposure to stress conditions *in vitro* was observed when compared to wild-type erythrocytes. Low to undetectable expression of hepcidin in *mk/mk* liver correlated with increased expression of growth differentiation factor 15 (GDF15) in the bone marrow and spleen.Conclusions: The phenotype of iron restricted erythropoiesis in spite of increased plasma iron present in 129S6/SvEvTac-*mk/mk* mice is reminiscent of that of DMT1-mutant patients but differs from originally described *mk/mk* mice strain MK/ReJ. In this regard, 129S6/SvEvTac-*mk/mk* mice seem to be more accurate model for comparison with patients' samples. The lack of tissue iron deposits in 129S6/SvEvTac-*mk/mk* is in contrast to hepatic iron overload of DMT1-mutant patients and likely reflects increased intestinal iron (heme or non-heme) absorption and redistribution of iron from erythroid tissues to the liver in the human subjects. Our results also show that impaired DMT1 function negatively affects all stages in the erythroid lineage.Grant support:Czech GrantAgency, grants No. P305/10/P210 and P305/11/1745; Internal Grant of PalackyUniversityOlomouc(LF_2012_016).

0943

SHORTER CAROTID ARTERY OCCLUSION TIME IN A THALASSEMIC MOUSE MODEL: A POTENTIAL ROLE FOR OXIDATIVE STRESS AFFECTING BOTH RBC AND PLATELETS

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Background. Beta-thalassemia is a common hereditary hemolytic anemia which is due to mutations in the alpha or beta-globin genes, resulting in partial or complete synthesis of HbA. Increased life expectancy due to improved therapy resulted in the appearance of complications in adult patients which were not recognized earlier due to hypercoagulable state. Among them were deep venous thrombosis pulmonary embolism as well as arterial thrombosis and stroke. **Aims.** Recently, we reported an increased platelet adhesion in patients with β thalassemia intermedia (Thromb Haemost. 2008 Nov;100(5):864-70) which may contribute to their thrombophilic state. The present study was carried out to elucidate the effect of thalassemic blood cells on the development of arterial thrombosis in carotid arteries in thalassemic mice. **Methods.** We used a model of transgenic heterozygous thalassemic mice (C57B1/6 Th3/+ , obtained from S. Rivella, Weill Medical College, NY,), that have targeted mutations in both murine beta globin genes (major and minor) on one chromosome. The mice exhibited enlarged spleens with average weight of 420 mg compared to 130 mg in the controls, associated with anemia and aberrant RBC morphology comparable with that found in patients with beta-thalassemia intermedia. Platelet counts were normal. There mice and their wild-type littermates were subjected to photochemical injury, and blood flow was monitored. **Results** :Mean time to occlusive thrombus formation of the carotid arteries in wild-type mice was 64.6±8 minutes compared with 49.1±9 minutes in thalassemic mice (n=10 per group; P<0.05). Flow cytometry measurement of oxidative stress markers revealed higher reactive oxygen species generation (mean fluorescence index (MFI) of 315 vs. 193 in controls, lower content of reduced glutathione (MFI of 34 vs. 128 in controls) and higher expression of phosphatidylserine (10.2% vs 7.6%), known to be associated with platelet activation, in platelets of thalassemic mice compared to wild type mice. Comparable results were obtained with RBC. These differences were unaffected by the application of either the photosensitizer or the laser beam in the injury model. Aspirin treatment (100mg/kg) delayed the time to occlusion in thalassemic mice, bringing it to a value close to wild type mice (57.8±8 minutes).**Conclusion**: The present study contributes to the identification of mechanisms and consequences of RBC pathology and platelet hyper- reactivity in thalassemic mice associated with increased parameters of oxidative stress. It is suggested that anti-platelet aggregants as well as anti-oxidants could be used for prevention of thrombosis in patients with β thalassemia intermedia.

0944

REFERENCE INTERVALS FOR HEMOGLOBIN IN SUBJECTIVELY HEALTHY ELDERLY INDIVIDUALS - HOW VALID ARE THE WHO CUT-OFFS? INSIGHTS FROM THE WWW.SENIORLABOR.CH STUDY

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Background. Anemia is a frequently encountered disorder in the elderly. The defining cut-offs are provided by the WHO and are 120 g/L for women and 130 g/L for men. Although older age is associated with lower hemoglobin levels, these cut-offs do not account for age. **Aims.** To characterize reference intervals in subjectively healthy individuals and to gain insights on factors being associated with hemoglobin levels. **Methods.** TheSeniorlabor study has so far recruited over 1600 subjectively healthy Caucasian inhabitants>60 yrs of age.Venous blood samples were drawn after an overnight fasting period. Hemoglobin was assessed using an XE-5000 hematology analyzer (Sysmex-Digitana, Switzerland). Reference intervals were assessed according to the CLSI-guideline C28-A3.*Results.* Here we report on the results from 557 men and 722 women. Men had significantly higher median hemoglobin levels than women(149 vs. 137 g/L; p<0.001), and there was a significant Spearman rank correlation between age and hemoglobin level (r=-0.113; p<0.001). Thus reference ranges were stratified according to age and gender. Double-sided 95% reference intervals for men were: 129-168 g/L (age 60-69), 125 - 168 g/L (age 70-79), 118-169 g/L (age >80). The respective reference intervals for females were: 120-157 g/L (age 60-69), 116 - 156 g/L (age 70-79), 115-164 g/L (age >80). A linear regression model incorporatingage, female gender, testosterone,ferritin, CRP, low ALAT, cholinesterase, and total bilirubin as significant predictors (all p<0.01) of hemoglobin concentrations accounted for 37.4% of hemoglobin concentration variance. Interestingly, in this model , we could not observe a significant association between hemoglobin and kidney function (CKD-EPI eGFR), folate concentrations, Holo-Transcobalamin concentrations, and fT4 concentrations.**Conclusions.** With progressive age, there is a considerable decline of the lower limits of hemoglobin concentrations., which is more pronounced in men than in women and seems to converge above age 80. In both sexes, the upper limit remains more or less constant over the whole age range. The WHO cut-offs seem to be valid at age 60, but tend to get too restrictive at older age. This may cause overdiagnosis of anemia, and consequently, may perturbate an elderly persons wellbeing and lead to unnecessary and costly follow-up investigations.

0945

CREB1 IS REQUIRED FOR HYDROXYUREA-MEDIATED INDUCTION OFGAMMA-GLOBIN EXPRESSION IN K562 CELLS

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Background. Hydroxyurea (HU) is a drug used for the treatment of hemoglobinopathies. HU functions by up-regulating γ-globin transcription and fetal

hemoglobin (HbF) production in erythroid cells. The K562 erythroleukemia cell line is widely used as a model system to study the mechanism of γ-globin induction by HU. However, transcription factors required for the up-regulation of γ-globin expression by HU in K562 cells have not been identified. Similarities between the HU and sodium butyrate (SB) pathways suggested CREB1 as a potential candidate. **Aims.** The aim of this study was to investigate the possible role of CREB1 in the HU / γ-globin induction pathway in K562 cells. **Methods.** We performed transient and stable RNAi experiments to show that CREB1 is necessary for HU-mediated induction of γ-globin expression and hemoglobin production in K562 cells. Furthermore, Western blot analyses demonstrated that CREB1 becomes phosphorylated in a dose-dependent manner after HU treatment of K562 cells. We also investigated role of a Gγ promoter CREB1 response element (G-CRE) in this pathway. First, quantitative ARMS-PCR (Amplification Refractory Mutation System) experiments were performed to demonstrate that HU induces both Gγ and Aγ expression in this cell line. In addition, electrophoretic mobility shift assays (EMSAs) were used to show that levels of CREB1 complexes binding to the G-CRE site are increased upon HU treatment and are decreased in the CREB1 knockdown cells. Conclusions. Our results suggest that CREB1 is necessary for γ-globin induction by HU in K562 cells"'a role which may, in part, be mediated through the G-CRE element.

0946

REFERENCE VALUES FOR ORAL IRON ABSORPTION OF BIVALENT IRON IN HEALTHY VOLUNTEERS

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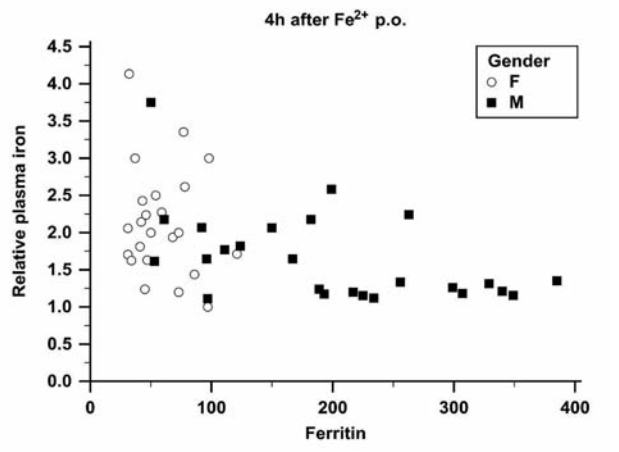
Background. Iron deficiency anaemia is the most frequent cause of anaemia in the western world. Despite progress in diagnosis and treatment there is a challenge in establishing the cause of iron deficiency in the absence of obvious reasons such as blood loss or deficient dietary intake. There are no prospectively validated iron absorption tests that could confidentially confirm or exclude iron malabsorption. **Aims**. To establish an oral iron absorption test and to define reference values of plasma iron in healthy volunteers after oral iron intake. **Methods.** Healthy volunteers were included into this study that was approved by ethics committee. Relevant comorbidities (inflammation, preexisting malabsorption or liver disease) were excluded by medical history and laboratory tests (haematogram, ferritin, CRP, ASAT). Each of the 49 volunteers received 200 mg of the bivalent ferrous fumarate in fasting condition at 8 AM after blood samples were drawn for plasma iron, ferritin, soluble transferrin receptor, haemoglobin, reticulocytes and reticulocyte haemoglobin. Two and 4 hours later as well as on days 3 and 5 plasma iron, haemoglobin, reticulocytes and reticulocyte-haemoglobin were measured, all of those in fasting condition. After a wash-out phase of two weeks 23 healthy volunteers proceeded to receive trivalent iron hydroxide polymaltose and 11 healthy volunteers did not receive any iron. In these latter two groups the identical laboratory analyses were performed as described above. Data are shown as mean ± standard deviation. Two sided t-test was used for comparison.



Results. Forty-nine healthy volunteers (24 females, 25 males; age 36±10 years) were studied. Haemoglobin was 136±7 g/L in females and 157±8 g/L in males; ferritin was lower in females (59±24 µg/L) compared to males (199±99 µg/L; p<0.0001); and fasting plasma iron was 17±4 µmol/L in females and 21±7 µmol/L in males. Plasma iron increased to 27±7 µmol/L in females and 28±9 µmol/L in males after 2 hours and to 34±12 µmol/L in females and 32±11 µmol/L in males after 4 hours (both p<0.0001 compared to iron values prior iron ingestion). All other parameters did not change relevantly. Relative increase of plasma iron after 4 h was higher in females (2.1±0.7) compared to males (1.7±0.6; p=0.02). While a negative correlation between ferritin and relative iron increase could be detected in males (r= -0.51, p=0.008), females had lower ferritin values in general and a wider variation of iron increase (see Figure). On days 3 and 5, we found values for all tested parameters comparable to initial values. There was no relevant increase of plasma iron after ingestion of trivalent iron in fasting condition or in volunteers not receiving iron. **Conclusions**. Reference values of absolute and relative iron increase after oral iron ingestion could be established. There was a significant increase of plasma iron after bivalent iron intake within 4 hours after ingestion. Relative iron increase correlates to iron storage. Females had in general lower ferritin levels and thus higher increase of plasma iron. These results lead the way to a prospective validation of an oral iron absorption test using bivalent iron among patients.

0947

ASSOCIATION OF CD40L IN PLATELET-MEDIATED INFLAMMATION IN SICKLE CELL DISEASE

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Background. The interaction between inflammatory cytokines, activated blood cells and endothelial cells is a critical step in vaso-occlusive processes in sickle cell disease (SCD), leading to vascular dysfunction, inflammation and additional recruitment of inflammatory cells to the vessel wall. It is now recognized that platelets (PLTs) modulate immuno-inflammatory reactions through cytokine secretion and subsequent interaction with leukocytes and endothelium. Platelet-associated CD40 ligand (CD40L), when bound to the CD40 receptor, plays an important role in this inflammatory response between platelets and other cells. **Aims.** Since the involvement of platelets in the vaso-occlusive process is not well understood, we hypothesized that CD40L may contribute to platelet-mediated inflammatory responses in SCD. This study evaluated the production of platelet-derived CD40L and the expression of its receptor on platelets and neutrophils of SCD individuals. **Methods.** IL-8, soluble ICAM-1, VCAM-1 and CD40L (sCD40L) were determined in PLT-free plasma/supernatant of stimulated (ADP or Collagen) and unstimulated PLTs ($2x10^8$/ml in Kreb's buffer) from healthy individuals (CON) and SCD patients by ELISA. Quantitative Real Time PCR was performed to analyze the CD40 gene expression in neutrophils from the peripheral blood of SCD and CON individuals. The expression of CD40 and P-selectin, and PAC-1 binding (anti-activated αIIbß3 integrin) on the PLT surface was determined by flow cytometry. **Results.** sCD40L was elevated in the plasma of SCD individuals (724.4±55.7pg/ml; n=90), compared to CON (241.5±34.6pg/ml, P<0.0001; n=41). Plasma sCD40L presented correlation with PLT counts in SCD individuals (r_s=0.255, P=0.015). No correlation was found between plasma sCD40L and IL-8, ICAM-1 or VCAM-1. PLT sCD40L release from SCD and CON (90min, 37ºC, 5% CO_2) were evaluated and PLTs of SCD patients released higher amount of CD40L (8347.0±1464.0pg/10^8 PLTs; n=10) than PLTs of CON individuals (3652.0±568.3pg/10^8 PLTs; n=5, P=0.019). sCD40L release from SCD PLT was augmented by incubation with collagen (P<0.001), but not ADP, indicating that collagen stimulation may be an important activator of CD40L release from platelets in SCD. Expression of CD40 receptor on the platelet surface was elevated in the SCD group, compared to the CON, and this expression presented a correlation with PAC-1 (r_s=0.498, P=0.018), a marker of PLT activation. Gene expression of CD40 protein (0.021±0.003U.A., n=14), was increased in SCD neutrophils, compared to CON (0.007±0.002U.A., n=9; P=0.005). **Conclusions.** The pro-inflammatory protein CD40L is elevated in the plasma of SCD patients and its release from SCD PLTs is higher when compared to CON PLTs. Several studies have reported that CD40L is a potent inductor of adhesion molecules and pro-inflammatory cytokines in endothelial cells and leukocytes, however no correlation was found between plasma levels of CD40L, IL-8, ICAM-1 and VCAM-1. We found an increase of CD40 expression in SCD PLT and this expression was correlated with PLT activation. Interestingly, the expressions of both CD40/CD40L on platelets could allow crosstalk between platelets, vascular cells and leucocytes. Gene expression of CD40 was also increased in SCD neutrophils, suggesting a possible participation of platelet-derived cytokines in activation of these cells. These results suggest that CD40L may play an important role in platelet-mediated inflammatory responses in SCD.

0948

GROWTH DIFFERENTIATION FACTOR 15 EXPRESSION AND REGULATION DURING ERYTHROID DIFFERENTIATION IN NON-TRANSFUSION DEPENDENT THALASSEMIA SYNDROMES

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Background. Growth differentiation factor 15 (GDF-15), a cytokine member of the TGF-beta superfamily that is released by mature erythroblasts, was shown to regulate hepatic expression and release of the iron regulatory protein hepcidin probably in response to increased iron demand from the bone marrow. However, the exact mechanisms regulating GDF-15 expression remain unknown. GDF-15 serum levels in non-transfusion dependent thalassemia (NTDT) patients are considerably elevated; however, little is known about its expression and regulation during thalassemic erythropoiesis. NTDT represents an ideal model to study thalassemic erythropoiesis and its relationship with iron metabolism. **Aims.** To determine the GDF-15 expression and regulation during normal and thalassemic erythroid differentiation in vitro. **Methods.** After informed consent, $CD34^+$ cells were obtained from peripheral blood of healthy volunteers (control group) and NTDT patients and cultured for 14 days with a medium stimulating erythroid differentiation. GDF-15 expression was evaluated at day 0 (erythroid progenitors), 7 (proerythroblasts) and 14 (mature erythroblasts) of culture by real-time PCR (2^-dCt). GDF-15 levels in culture supernatants were evaluated by ELISA assay (R&D Systems, Minneapolis, MN). Intracellular iron concentrations were estimated by colorimetric assay (BioVision, Milpitas, CA).

Table 1

	GDF-15 expression (2^-dCt)			GDF-15 levels (pg/mL)		Iron concentrations (µM)	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Control	0,005 ± 0,004	0,04 ± 0,03	5,66 ± 2,64*	198 ± 49	5595 ± 4245*	28 ± 2,8	9,5 ± 4,5*
NTDT	0,18 ± 0,04°	0,67 ± 0,66°	4,5 ± 3,6*	636 ± 415°	8427 ± 5136*	13,5 ± 3,6°	5,8 ± 3,4*

* day 14 vs day 7: p<0.05; * NTDT vs control: p<0.05

Results. GDF-15 expression and secretion increased significantly during erythroid differentiation (from day 7 to day 14 of cultures) both in control and NTDT cultures. However, GDF-15 levels at the early phase of differentiation (day 0 and day 7) were significantly higher in NTDT cultures compared to controls. Intracellular iron concentrations significantly decreased during erythropoiesis in both control and NTDT cultures; however, at day 7 iron levels were significantly lower in NTDT cultures compared with controls (Table 1).**Conclusions.** GDF-15 expression and levels in erythroid cultures are related to the erythropoietic stage of differentiation, being higher in mature erythroblasts. However, at the early phase of erythropoiesis, GDF-15 expression and secretion in NTDT cultures is higher than controls, reflecting the higher erythropoietic activity of thalassemic cells. There is an association between GDF-15 levels and intracellular iron concentrations, with lower concentration being associated with higher GDF-15 production. These findings suggest that GDF-15 can be considered a marker of erythropoietic activity and that intracellular iron concentrations could be a major contributor to GDF-15 regulation, although other factors, such as oxidative stress, can still be involved in this process.

0949

EVALUATION OF FERRIC CARBOXYMALTOSE FOR REDUCTION OF DOXORUBICIN-INDUCED CARDIOTOXICITY IN IRON DEFICIENT SPONTANEOUSLY HYPERTENSIVE STROKE PRONE RATS

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Background. Iron deficiency anemia (IDA) and hypertension are frequent complications and cardiac risk factors in patients with neoplasms and cytotoxic therapies. IDA can induce oxidative, nitrosative and inflammatory stress. Furthermore, previous studies demonstrated that IDA can aggravate doxorubicin (DOX)-induced cardiotoxicity in spontaneously hypertensive stroke prone (SHRSP) rats. **Aims.** This study evaluated whether ferric carboxymaltose (FCM) treatment can prevent IDA- and DOX-associated decrease of cardiac performance and induction of oxidative and nitrosative stress as well as inflammatory reactions in SHRSP rats. **Methods.** SHRSP rats were randomized into five groups (G1-G5; n=8 each). IDA was induced in groups G2-G5 by a 12-week low iron diet; G1 had normal diet (control). G3-G5 received DOX (4 mg/kg body weight/week) for 6 weeks. G4 and G5 received one dose of FCM (15 mg iron/kg body weight) either concomitantly with or 3 days after the second DOX administration. In G1-G3, active treatments not applicable to the group were replaced with saline solution. Fractional shortening (FS; difference between the end-systolic and end-diastolic dimension normalized by the end-diastolic dimension) was assessed by echocardiography before initiation of DOX treatment (baseline [BL]) and at the end of the study (EOS). Further analyses at the end of the study assessed hemoglobin (Hb) levels and transferrin saturation (TSAT) in blood samples and markers of oxidative, nitrosative and inflammatory stress (thiobarbituric acid reactive substances [TBARs], reduced/oxidized glutathione ratio [GSH/GSSG]; nitrotyrosine; tumor necrosis factor-alpha [TNF-α], interleukin-6 [IL-6]) in homogenates and tissue sections from heart samples.

Table	G1 control	G2 IDA	G3 IDA/DOX	G4 +FCM (c)	G5 +FCM (d)
Hb (g/dL)	17.2±0.7	11.0±0.3*	10.3±0.3†	15.6±0.4‡	15.5±0.6‡
TSAT (%)	41.3±2.2	20.0±3.2*	19.2±3.1*	36.2±2.0‡	36.4±2.1‡
FS (%, BL)	45.4±1.3	40.6±1.2*	41.0±0.9*	41.1±1.0*	41.3±1.1*
FS (%, EOS)	44.8±1.1	35.7±0.6*	32.2±1.0†	38.7±0.6‡	39.2±0.9‡
TBARs (nmol/mg)	1.8±0.2	2.5±0.2*	4.3±0.2†	2.9±0.1‡	2.7±0.2§
GSH/GSSG (ratio)	4.8±0.2	3.9±0.1*	2.4±0.2†	3.6±0.2‡	3.8±0.2§
Nitrotyrosine (%/area)	11.6±1.3	16.8±3.8*	29.1±3.4†	16.9±3.1§	16.1±2.2§
TNF-α (%/area)	11.9±1.1	15.0±1.5*	19.6±1.4†	14.2±1.2§	14.1±1.3§
IL-6 (%/area)	3.6±1.0	20.3±1.6*	28.4±2.5†	15.2±1.4‡	14.4±1.6‡

mean±SD, (c) concomitant with DOX, (d) delayed 3 days after DOX
*p<0.01 vs. G1; †p<0.01 vs. G1,G2; ‡p<0.01 vs. G1-G3; §p<0.01 vs. G1,G3

Results. Iron deficient rats treated with a single dose of FCM in addition to a 6-week DOX regimen (G4-G5) maintained significantly better cardiac performance (FS) compared to saline-treated iron deficient rats with or without DOX (G2, G3) (Table 1). In fact, FCM maintained FS close to baseline levels. Addition of FCM to the DOX treatment also lead to significantly less oxidative, nitrosative and inflammatory stress compared to saline-treated, DOX-exposed, iron deficient rats (G3), thus reducing the synergistic effects of IDA and DOX (G2 and G3 vs. G1). Notably, the treatment benefit was independent whether FCM was given together with DOX or 3 days afterwards. **Conclusions.** A single dose of FCM in the early phase of a 6-week DOX treatment schedule prevented cardiac dysfunction and alleviated DOX- and IDA-associated oxidative, nitrosative and inflammatory stress in the heart of SHRSP rats. Concomitant or delayed administration of FCM showed the same benefit in reduction of DOX-induced cardiotoxicity and evaluated stress parameters.

0950

DECREASED AQUAPORIN 1 (AQP1) EXPRESSION AND LOW SERUM OSMOLALITY IN HEREDITARY SPHEROCYTOSIS (HS)

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Background. AQP1 is the membrane water channel responsible for fast changes of red cells volume as response to tonicity of medium. The red blood cell membrane is extensively remodeled during erythropoiesis, conditioning the expression of membrane proteins. Throughout the process of enucleation, proteins are distributed between the extruded nuclei and the remaining reticulocyte. As the aberrant distribution of proteins in HS generates deficiencies of proteins other than those codified by the mutated gen, we postulated that AQP1expression might be impaired in HS erythrocytes. Moreover, since AQP1 expression in the mature red cell is modulated by serum osmolality, we also measured serum osmolality of HS patients. **Aims**. To evaluate AQP1 expression and impact of serum osmolality on AQP1 expression in patients with HS **Methods.** AQP1 expression was evaluated through flow cytometry in 6 nor-

mal controls (NC), 1 autoimmune hemolytic anemia, 10 HS (2 mild, 3 moderate, 2 severe, and 3 splenectomized), and 3 silent carriers (SC). Fixed and permeabilized red cells were stained with rabbit anti-AQP1 antibody followed by FITC or Phycoerythrin conjugated anti-rabbit IgG. The number of AQP1 molecules per erythrocyte was cuantified. The effect of AQP1 inhibitors (Cl2Hg 0.1-40 □M and Cl2Cu 400 □M) was evaluated through water flow-based tests: osmotic fragility (OF) and hypertonic cryohemolysis (CH). Serum osmolality was measured using a pressure vapor osmometer in 20 NC and 13 HS (4 mild, 4 moderate, 2 severe, and 3 splenectomized). HS and SC individuals presented single or combined deficiencies of ankyrin, spectrin, protein 4.1, and protein 4.2 **Results.** AQP1 expression was decreased, compared to simultaneously performed NC, in all of the HS patients and SC. The decrease was greater as HS was more severe: significant correlation between AQP1 expression and hemoglobin level was found (rho Spearman: 0.6510; p; 0.0086). Negative correlationship between AQP1 expression and CH was observed (n=16; r2=-0,607; p:0,006). AQP1 inhibitors increased CH but did not modify OF. The CH increase induced by Hg++ showed a direct positive relationship with the level of AQP1 expression (n=4, r2= 0,916). Osmolality was significantly lower in HS patients compared to NC (270.5±1.58 vs. 275.9±1.50, respectively; p: 0.015) **Conclusions**. Decreased AQP1 expression reveals a deficiency common to all patients with HS, a disorder based on alterations of membrane proteins and cytoskeleton. It is likely that an abnormal AQP1 secretion occurs during the enucleation process. As reticulocytes of HS patients are immersed within plasma with lower osmolality than normal, the loss of AQP1 might be increased during the subsequent reticulocyte maturation process. We suggest that these two mechanisms could help to explain these observations. Results of assays with inhibitors suggest that AQP1 decrease influences processes involving water efflux rather than water influx. No study concerning these findings has been published in the literature up to date.

0951

RED BLOOD CELL PYRUVATE KINASE (PK) HYPERACTIVITY UNLINKED TO THE PKLR OR PKM2 GENE - PREDICTION OF A NOVEL ELEMENT REGULATING PKLR EXPRESSION

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Background. Red blood cell (RBC) pyruvate kinase (PK-R) is an important enzyme of RBC energy metabolism, yielding ATP in the final step of glycolysis. Hereditary deficiency of PK-R is a autosomal recessive disorder associated with chronic nonspherocytic hemolytic anemia. It is due to mutations in *PKLR*.PK hyperactivity is a very rare autosomal dominant abnormality of RBC metabolism associated with high ATP levels and mild erythrocytosis. In the few families examined the hyperactivity has been attributed to either persistent expression of the PK-M2 isozyme (Max-Audit et al. Blood 1980;56:902) or a Gly37Glu missense mutation in *PKLR* (Beutler et al. Hum Mutat 1997;9:282). **Aims.** To identify the nature and origin of a novel case of PK hyperactivity. **Methods.** *PKLR* analysis was performed by DNA sequence analysis and Multiplex Ligation-dependent Probe Amplification (MLPA). Enzymatic characterization of PK-R was performed by standard **Methods.** RBC PK expression was studied by western blot using antibodies against PK-L and PK-M2. PK-R antigen levels were determined by ELISA. **Results.** We identified PK hyperactivity (34.3 U/gHb; reference range: 6.1-12.3 U/gHb) in a 2-year-old female child who was also heterozygous for glucose-6-phosphate dehydrogenase (G6PD) deficiency. The mutant G6PD (G6PD Vanua Lava) was inherited from the child's mother whereas the child's father, paternal grandfather, paternal aunt and younger sister all showed elevated PK activity (range 20.5-30.4 U/gHb) in absence of reticulocytosis. The mother and paternal grandmother showed normal PK activity. All family members were clinically unaffected.DNA sequence analysis failed to detect any mutation in the coding region of *PKLR*, including splice sites, or 1000 bp of its upstream regulatory region. Furthermore, MLPA analysis showed no variations in *PKLR* copy number. In agreement with these results PK-R displayed no abnormal kinetic properties: K_m for phosphoenolpyruvate and ADP, as well as inhibition by ATP were all normal. Western blot analysis of PK-M2 expression showed very low but similar amounts of the fetal isozyme in all family members. PK-R expression, in contrast, was markedly elevated in all family members displaying PK hyperactivity. Further quantification of PK-R expression by ELISA demonstrated antigen levels that varied between 998-1601 arbitrary units (au)/gHb in affected individuals (mean: 1324 au/gHb), whereas the 494 and 789 au/gHb as detected in the 2 unaffected individuals was comparable to the normal range: 171-711 (mean 441) au/gHb. Hence, the 2-3 times elevated PK activity was accompanied by a concomittant increase in protein levels. **Summary and Conclusions.** We have identified a family with a novel type of inherited PK hyperactivity. The autosomal dominant abnormality was not due to an altered property of PK-R or persistent expression of the PK-M2 isozyme but, rather, was caused by isolated overexpression of the PK-R isozyme. We therefore postulate the existence of a novel, yet-unidentified, DNA region regulating *PKLR* gene expression. We expect that mutations of this region may be involved in patients with PK deficiency without mutations in *PKLR*. In addition, this region may represent one of the putative phenotypic modifiers involved in the highly variable expression of PK deficiency

0952

IRON BURDEN, LOW GRADE INFLAMMATION, ENDOTHELIAL DYSFUNCTION AND ANGIOGENESIS IN PATIENTS WITH THALASSEMIA INTERMEDIA

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Background. Placental growth factor (PlGF) is a member of the VEGF family of angiogenic proteins which binds to its receptor, the fms-like tyrosine kinase receptor 1 (Flt-1 or VEGF-R1). These growth factors exert pleiotropic effects, potentially beneficial, such as the promotion of angiogenesis, and/or potentially harmful pro-inflammatory effects, such as the promotion of endothelial dysfunction and pulmonary hypertension. von Willebrand factor (vWF) has been proposed as a biomarker of endothelial damage/dysfunction because increased plasma levels have been found in inflammatory and atherosclerotic vascular diseases and is defined as a novel link between hemostasis and angiogenesis. **Patients and Methods.** Using peripheral biomarkers, we investigated whether alterations in inflammation, endothelial dysfunction and angiogenesis relate to iron overload in patients with thalassemia intermedia (TI). Thirty-four adult patients with TI were included in the study, while 20 healthy individuals served as controls. We measured by means of immunoenzymatic techniques automated and/or manual, serum levels of high-sensitivity C-reactive protein (hs-CRP), von Willebrand factor, nitric oxide (NO) along with PlGF and soluble Flt-1, and ferritin in patients and controls. **Results.** The main results of the study showed that: a) plasma levels of vWF, NO, PlGF, sFlt-1 and ferritin were significantly higher in patients with TI compared to controls (88.0±21.8 vs 71.1±21.5 IU/dL, 101.5±34.7 vs 52.1±8.2 mmol/L, 52.2±20.0 vs 17.2±4.0 pg/mL and 96.5±25.2 vs 76.8±11.5 pg/mL, 740.5±505.2 vs 72.0±34.5, respectively ($p<0.01$), while angiogenic balance expressed as sFlt-1/PlGF was significantly lower in patients with TI compared to controls ($p<0.0001$), b) ferritin levels showed significant correlation with hs-CRP ($r=0.485$, $p<0.01$), vWF ($r=0.510$, $p<0.008$), NO ($r=0.535$, $p<0.005$), PlGF($r=0.495$, $p<0.01$) and sFlt-1($r=0.385$, $p<0.05$). **Conclusions**. Our findings demonstrate that patients with TI have a significant degree of low grade inflammation, endothelial dysfunction and active angiogenesis. The severity of these events seems to be related to the degree of ferritinemia, suggesting a possible pathogenetic role for iron load.

0953

SETTING UP SERUM HEPCIDIN ASSAY IN PEDIATRICS

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Background. The measurement of circulating hepcidin is considered a promising biomarker for evaluating the iron status in various diseases. Very few commercial tests are available and assay harmonization for hepcidin has not been reached as yet, making reference intervals and consequent clinical decisions still elusive for each assay and specific population. In addition very rare studies have been conducted in children so far both on reference values and on correlation with age, sex and iron status in different clinical subsets. **Aims.** The aim of this study was to set up hepcidin testing in children by using a commercially available kit with automated and high throughput procedures. In addition we aimed to clinically validate the test for use in paediatric age. **Methods.** One hundred and thirty four subjects were included in the study. Control group included 86 healthy children while the patients' group comprised 52 patients (19 with anemia in systemic Juvenile Idiopathic Arthritis (JIA), 14 with b-thalassemia major in chronic transfusion and chelation regimen, 19 with iron deficiency anemia (IDA) mostòy on iron supply. A subset of 8 patients with systemic JIA were considered before or after treatment with anakinra. All the patients were tested for hematologic (Hb, MCV, hematocrit, serum iron, ferritin, transferring) and inflammatory (CRP, ERS and IL-6) parameters by using routinary laboratory techniques. Hepcidin-25 was measured by using a commercially available ELISA kit with automated procedures. **Results.** In control subjects, hepcidin levels showed a normal distribution (mean =40.8 ng/mL , SD=13.9) and were significantly higher in males (median: 43.6; 1^{st}-3^{rd} q: 32-52.7) than in females (median: 36.4; 1^{st}-3^{rd} q: 28.5-45.7) (P=0.039). In anemia of systemic JIA, hepcidin concentrations were significantly higher than in controls. After treatment with anakinra, an anti IL-1 agent hepcidin levels decreased significantly, and so

did inflammatory markers. Hepcidin in b-thalassemic and IDA patients was comparable to normal subjects. Correlations between hepcidin concentration and other iron parameters was investigated in both control and in the patient groups. No significant correlations were observed in controls while in the whole group of patients a significant inverse correlation could be found between hepcidin serum levels and age (r_S= -0.40), MCV (r_S= -0.40), serum iron (r_S= -0.55) and plasma transferrin saturation (r_S= -0.54). In thalassemic patients an inverse correlation between hepcidin and age (r_S= -0.51), most of red cells parameters, serum iron (r_S= -0.53) and tranferrin levels (r_S= -0.42) was observed. In systemic JIA patients only a moderate positive (R= 0.48) correlation could be seen between hepcidin and transferrin (r_S= 0.48). In iron deficiency anemia (IDA) none of the correlations reached the level of 0.40. **Summary and Conclusions.** Our study provides a reliable technical evaluation of the normal range values of serum hepcidn in childhood. In addition it shows that hepcidin serum level may discriminate microcitic inflammatory anemia of JIA from IDA and may be investigated as a potential marker of good chelation in thalassemic patients. Overall it may a helpful tool for addressing future studies aiming to further understand the role of hepcidin in managing iron disorders in children.

0954

FINE-MAPPING OF TELOMERIC BREAKPOINTS OF CHROMOSOME 16P DELETIONS IN ALPHA-THALASSEMIA OR ATR-16 PATIENTS

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Background. Alpha-thalassemia is a genetic disorder of hemoglobin, which is a protein in the erythrocytes responsible for oxygen and carbon dioxide transport. Approximately 80% of all alpha-thalassemias is caused by deletions in the alpha-globin gene cluster located on chromosome 16p13.3. The majority of the deletions involves one or both alpha-globin genes and can be detected by gap-PCR for the 7 most common deletions during routine diagnostics. However, larger deletions also occur in this region causing alpha-thalassemia. When the deletion length is more than 1.5 Mb, they may be associated with a variable clinical picture, including alpha-thalassemia, dysmorphic features and mental retardation, which is known as the alpha-thalassemia-mental retardation syndrome (ATR-16). Most of these deletions extend a considerable end into the 3' direction, either involving the complete telomeric end of chromosome 16p, or leaving the telomeric region intact. **Aims.** The aim of this study was to investigate different types of telomeric deletions on chromosome 16p, leading to alpha-thalassemia or ATR-16. By determining the breakpoints of the deletions more exactly, it is possible to study the molecular mechanisms underlying these rearrangements. Furthermore, when breakpoints are determined at sequence level, relatively simple gap-PCR assays can be designed for diagnostic purposes. **Methods.** A group of 25 patients carrying a deletion on chromosome 16p, extending into the telomeric region, was investigated. These deletions were detected by MLPA during routine diagnostics for hemoglobinopathy. Fine-tiling array CGH was applied to fine-map the deletion breakpoint more precisely. In cases with an intact telomeric region, primers were designed to perform gap-PCR and sequence analysis to determine the exact breakpoints. **Results.** From the 25 cases studied, the fine-tiling array CGH showed an intact telomeric region in 13 cases. The other 12 patients carried deletions involving the whole telomeric end of chromosome 16p. Gap-PCR and sequence analysis to determine the exact breakpoint was successful in two cases. **Conclusions.** The fine-tiling array CGH technology is a valuable tool to fine-map deletion breakpoints. In this study, the array showed to be able to discriminate between interstitial deletions with an intact telomeric region (telomere capture) and deletions involving the complete telomeric end (telomere healing). Furthermore, array CGH results enabled the design of gap-PCR assays for 2 deletions. Due to the repetitive nature of the telomeric sequence, it is very difficult to design unique primers in this region. However, determination of the 3' end breakpoint by aCGH also provides valuable information.Because many genes are located at chromosome 16p13.3, loss of this region may have significant clinical consequences such as the ATR-16 syndrome. It is yet unknown which genes are responsible for the mental retardation phenotype in these patients. Therefore, it is important to study this kind of deletions to gain more insight in the genotype-phenotype correlations.

0955

A NOVEL 506 KB DELETION CAUSING εγδβ THALASSEMIA

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Background. εγδβ-thalassemias are rare and found only in the heterozygous form. They are caused by large deletions in the β globin cluster and are divided into two groups: Type I deletes the β globin gene and all or most of the β globin cluster, and Type II deletes the upstream regulatory region (β LCR) but leaves the β globin gene itself intact. The εγδβ-thalassemia heterozygous adult phenotype is like that of β thalassemia trait but with normal HbF and HbA_2 levels. However, newborns can present with severe hemolytic anemia that require blood transfusions. **Aims and Methods.** Here we describe a novel εγδβ-thalassemia deletion in a Pakistani family, named Pakistani I. The characterization of the deletion has encompassed a multitude of methodologies, mirroring changing DNA analysis technology; Southern blot hybridization, quantitative PCR, MLPA and CGH array mapping, gap PCR and sequence analysis. The Pakistani I deletion is 506 kb in size and the second largest reported so far. The deletion removes the entire β globin cluster and a large region upstream. The 5′ breakpoint is located 431 kb upstream of the ε gene, and the 3′ breakpoint, 75 kb downstream of the ε gene. The regions flanking the deletion are both LINE/LTR repeats but there was no homology between the 5′ breakpoint region and the 3′ breakpoint region. The 3′ breakpoint junction includes a duplication and inversion of a 250 bp region within an LTR repeat. This is adjacent to a 160 bp palindrome, of which one half has been deleted. The 250 bp repeat and the palindrome share a short 9 bp common junction sequence at their ends which may have led to a recombination event. The region flanking the 3′ breakpoint also includes seven known SNPs and a novel 24 bp deletion. The 5′ breakpoint junction is a simple break point within a LINE repeat. **Conclusions.** To date, 25 εγδβ-thalassaemia deletions have been reported: 15 are type I and 10, type II. Deletions over 0.5 Mb causing εγδβ-thal are extremely rare, this deletion being only one of two, and the second largest reported so far. Whilst detection of deletions is becoming easier and quicker with the use of MLPA and array CGH, full characterization has only been achieved in a few of the deletions reported so far. This case illustrates the technical challenges of defining the exact breakpoints of deletions which occur within repetitive regions. For comprehensive prenatal genetic counseling, the importance of fully characterizing these deletions is as relevant today, as it was two decades ago, when characterization of this deletion first began. Since εγδβ-thalassaemia deletions tend to be unique, there is a need for a comprehensive single methodology, such as next generation sequencing, that can fully characterize breakpoints and recombination events in a single analytical process. By comparing breakpoints among different deletions we may gain further insight into the biological processes that create them.

0956

IMPROVEMENT OF GENETIC DIAGNOSIS IN α-THALASSEMIA PATIENTS: MLPA DETECTION OF ELEVEN ALTERATIONS IN THE αα GLOBIN CLUSTER (INCLUDING THREE NOVEL DELETIONS)

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Background. Copy number variations (CNVs) are very common events in the α globin cluster. In this cluster there are two functionally α gene copies (four copies per diploid genome, denoted as αα/αα). Ninety percent of α-thalassemia patients carry a large deletion in this genetic region. There are two kinds of deletions: α+ deletions eliminates only one α gene copy (-α/αα), and α0 eliminates both copies in the cluster (—/αα).α0 carriers are usually healthy individuals with mild microcytic anemia, however, since α-thalassemia is one of the most prevalent monogenic disorders around the world, their offspring are in risk of developing: 1) a thalassemia intermedia condition named HbH dissease (—/-α) or 2) Hb Bart's dissease (—/—), a lethal condition for the fetus (in the late fetal stage of development), with the consecuent pregnancy complications for the mother. Thus, screening for deletions in patients suspected to have thalassemia is mandatory in order to prevent these events, and there are PCR methods designed to detect specifically a few of the most common deletions in the cluster. Nevertheless, rare and novel deletions can not be detected with

such methods, and the molecular diagnosis becomes difficult in a considerable proportion of the cases.The recent development of CNV searching tools allow to detect both previosly known and novel deletions, helping to improve the genetic diagnosis in α-thalassemia patients.AIM:This is a descriptive work showing the results of the analysis by Multiplex Ligation-dependent Probe Amplification (MLPA) of cases suspected to carry a rare alteration in the α globin cluster.METHODS:Screening for most common deletions was carried out with Alpha Globin StripAssay method in α-thalassemia patients (microcytic hipocromic anemia and normal HbA2 levels). MLPA was performed in those patients with normal or inconsistent genetic results after the initial screening.The MLPA is a quantitative technique based on the amplification and a subsequently fragment analysis of multiple probes hybridized across a region of interest. This method allows for a genetic profile showing the copy number variation of those targets. Here we used a commercial kit (P140-B3 HBA, MRC-Holland) that contains 25 probes designed to detect copy number changes in the α cluster, from POLR3K gene to 4Kb downstream of α1 globin gene, spanning more than 130Kb.RESULTS:Eleven different genetic alterations have been detected in this study (detailed view in figure 1); three of them have not been previously described in scientific publications and are novel alterations.CONCLUSIONS:In some cases, StripAssay method leads to interpretation mistakes in genetic diagnosis. The reason for this is that the method is designed to detect just the eight commonest deletions worldwide. Thus, in carriers of a combination of a common α+ deletion (ie α3.7Kb) together with a rare α0 deletion (that can not be detected with this method), the former technique indicates a (-α/-α) condition while the real condition is a (-α/—) genotype.Within this scenario, MLPA analysis improves the molecular diagnosis and the genetic counseling in this group of diseases.

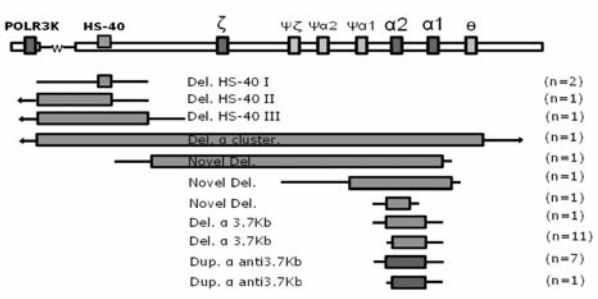

Figure 1. Alterations detected in this study. Bars denote the minimal lenght of the deletion/duplication, while lines show the theoretical maximun lenght. Arrows indicate that rearrangement can not be delimited at that point. Finally, the number of cromosomes (n) affected for each alteration is indicated in column at the right.

0957

A THREE BASE PAIR DELETION IN 3'UTR OF G6PD GENE : [3'UTR +66 DEL (-GGA)]

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Background. Glucose-6-phosphate dehydrogenase (G6PD, MIM 305900) catalyzes the first step in the pentose phosphate pathway and plays a critical role for protecting cells against oxidative stress. Molecular characterisation of the G6PD gene, showed that G6PD deficiency is mainly caused by: over 160 missense mutations leading to amino acid substitutions, eight base pair deletions (6 three, 1 six and 1 twenty four bases) that do not produce frame shifts and two splicing mutations Up to now, there is no documented mutation in the 5' or 3' untranslated region (UTR) of this gene. **Aims.** We aimed to check whether this deletion is causing G6PD deficiency or it is simply a polymorphism. **Methods.** Deletion was detected by PCR amplification followed by sequencing. Apart the two probands (unrelated females) in whom we found the deletion of 3 bp, we sequenced 150 chromosomes bearing different variants responsible for G6PD deficiency and 150 B chromosomes.Results. The deletion [3'UTR +66 del (-GGA)] was detected in two unrelated females in a heterozygous state. The two proband showed acute haemolysis, palpable spleen and history of neonatal jaundice.Exploration for hemoglobinopathies and pyruvate kinase were negative.The chromosomes B and those bearing deficient variants were negative for the deletion. We suspect that this deletion is the causative of G6PD deficiency, especially that ARN secondary structure changes when deletion occurs.Summary:In the herein study we report, for the first time, a 3-base pair deletion in the 3'UTR of the G6PD gene [3'UTR +66 del (-GGA)]. This deletion is in a heterozygote state. We suspect it is causative of G6PD deficiency.

0958

MODULATING FACTORS IN BETA-THALASSEMIA INTERMEDIA; THE ROLE OF COPY NUMBER VARIATION INVOLVING THE ALPHA-GLOBIN GENE CLUSTER

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Background. Recently we have described two cases of heterozygosity for the common β°-thalassemia mutation β39 (C>T) presenting with a thalassemia intermedia phenotype. Multiplex Ligation dependent Probe Amplification (MLPA) analysis of the α-globin gene cluster revealed two new rearrangements, consisting of a full duplication of the α-globin genes locus including the upstream regulatory elements. Here, we present three other cases. **Aims.** Study the molecular background. of carriers of a single beta-thal mutation expressing a beta-thalassemia intermedia phenotype. **Methods.** Multiplex Ligation-dependent Probe Amplification (MLPA), direct sequencing and haematological/biochemical analysis.**Results.** The first is a 2 year-old girl of mixed Sephardic Ashkenazi Jewish origin living in Canada, who presented with a pronounced microcytic hypochromic anemia. She was found to have borderline HbA_2 levels and heterozygosity for a β polyA mutation inherited from the parent who had mild microcytic hypochromic anemia due to heterozygosity for the same β-mutation. The second case, a 6 year old girl of middle-eastern origin living in the UK, has severe anemia and splenomegaly, while the mother from whom she has inherited the β^0-thalassemia mutation is clinically asymptomatic. The third case is an Albanese adolescent living in Rome with pronounced microcytic hypochromic anemia, borderline HbA_2 levels and heterozygosity for the β IVS1-110 g>a mutation. She showed modest hepatomegaly and marked splenomegaly, elevated bilirubin (~3mg%), normal ferritin (~160 ng/ml) and reticulocytosis (>4%). MLPA analysis of the α-globin gene cluster in the 3 cases revealed three new rearrangements, consisting of a full duplication of the α-globin gene cluster and the upstream regulatory elements, arranged head-to-tail in the patient's genome. **Summary and Conclusions.** We report the clinical and hematological data and the molecular characterization. We conclude that α-globin gene duplication is more common than previously thought and should be investigated as a contributing cause in all unexplained unusually severe anaemia in carriers for β thalassemia.

0959

HOMOZYGOUS ABSENCE OF THE TMPRSS6 GENE IN AN 11 YEARS OLD CHILD RESULTING IN SEVERE IRON DEFICIENCY IRON REFRACTORY ANEMIA

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Background. Iron Refractory Iron Deficiency Anemia (IRIDA) is a rare hereditary disease characterized by microcytic hypochromic anemia with low transferrin saturation (TSAT), not responsive to oral iron. The genetic substrate of IRIDA is the *TMPRSS6* gene (transmembrane protease serine 6) encoding the protein matriptase-2.*TMPRSS6* defects lead to increased levels of hepcidin, inappropriate for the low body iron status, resulting in decreased intestinal iron absorption and iron release by macrophages by degradation of the cellular iron exporter ferroportin.Hitherto reported human *TMPRSS6* defects cause impaired function of matriptase-2. Here we describe an 11 years old girl with a severe phenotype of IRIDA due to a homozygous "knockout"of *TMPRSS6.* **Aims.** Description of phenotype and genotype of homozygous absence TMPRSS6 mutations. **Methods. Case report. Results.** An 11 years old girl born to healthy consanguineous Northern African parents presented with severe microcytic anemia with low TSAT at the age of 3 years (Hb 7.9 g/dl, MCV 53 fl , TSAT 4%, ferritin 29 µg/l).Oral iron was not effective, in contrast to intravenous iron (Hb 12 g/dl, MCV 64 fl, TSAT 7%, ferritin 304 µg/l). There was no evidence of malabsorption , gastrointestinal blood loss or hemoglobinopathy.In addition to the anemia, the patient suffered from unclassified mental retardation (IQ around 50) and auditory hallucinations.Serum hepcidin, analysed by time-of-flight mass spectrometry, was 25.4 nmol/L, which is inappropiately high relative to the low TSAT (TSAT/hepcidin ratio 0.16 %/nM, local reference values for premenopausal women 2.0-330 %/nM, for children unknown) and suspicious of the IRIDA-phenotype.SNP analysis (2.7M cyto array Affymetrix) of the *TMPRSS6* gene showed a homozygous deletion of 120 kb at the distal breaking point in

intron 2, arr22q12.3 (35,704,120-35,827,974)x0, confirming the IRIDA-genotype. In addition to the matriptase-2 encoding region, 4 other not previously reported genes were deleted. Two of them might affect mitochondrial function. However, serum lactate, amino-acids and organic acids were within reference ranges.It is well possible that the retardation is the result of the severe lack of iron, as iron deficiency in childhood may have severe effects on neurodevelopment. An alternative explanation can be the absent genes.Screening of the family showed that both the parents and the brother were heterozygous for the relevant deletion. The mother and brother of the patient are asymptomatic with Hb, MCV and TSAT/hepcidin ratio within reference ranges. The father's phenotype is unknown. **Conclusions.** We conclude that a homozygous absence of the *TMPRSS6*-gene is compatible with life and results in severe IRIDA. We hypothesize that the iron deficiency in severe IRIDA may lead to mental retardation.Our observations support the idea that IRIDA is an autosomal recessive disease. However, anecdotal reports of symptomatic heterozygous IRIDA patients suggest a co-dominant disease, indicating that the inheritance of IRIDA still needs elucidation.Intravenous iron is effective in IRIDA. A dose limiting side effect is toxic macrophage iron loading. Recent reports suggest a positive influence of erythropoetin by creating a better iron utilization. The optimal treatment of IRIDA remains to be investigated.

0960

LYN DEPENDENT PHOSPHORYLATION OF BAND 3 REGULATES ERYTHROCYTE FUNCTIONALITY

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Background. Optimal *ex vivo* or *in vitro* expansion of erythrocytes is important if red cell production is to be realized on a grand scale for transfusions. The flexibility and rigidness of the erythrocytes is in part dictated through associations of the spectrin based cytoskeleton with the plasma membrane. Within the abundant band 3 macro-complexes within the erythrocyte membrane, the interaction of tetrameric band 3 with ankyrin and protein 4.2 is of pivotal importance to function as a spectrin cytoskeleton-membrane anchoring point. This interaction, in part, dictates the shape of erythrocytes. The strength of this cytoskeleton-membrane association may thus regulate membrane flexibility and shape of erythrocytes. This could be important to withstand shear stress in the capillaries and during band 3-spectrin complex formation throughout reticulocyte maturation to biconcace erythrocyte. Band 3, ankyrin, protein 4.2 as well as spectrins have been shown to be tyrosine and serine phosphorylated and this phosphorylation could potentially regulate the strength of cytoskeleton-membrane association. **Aims.** How does tyrosine phosphorylation of band 3 influence the functionality of erythrocytesMethods:To understand the role of phosphorylation in erythrocyte flexibility, phosphorylation is induced by different concentration of ortho-vanadate treatment (OV). The effect of this treatment on morphology (microscopy), osmotic tolerance (NaCl concentration curve), deformability (ARCA) and cytoskeleton-membrane linkage (detergent extractability assays) is assayed. We use immune-precipitations to asses how phosphorylation affects the associations with the band 3 macrocomplex.Results. We found that 0.1mM ortho-vanadate (OV) treatment causes increased fragility of erythrocytes and an echinocyte morphological appearance. Furthermore, orthovanadate treatment results in band 3 tyrosine and serine phosphorylation. This phosphorylated form of band 3 is extractable by detergents indicating a weakened cytoskeleton link (C12E8), whilst spectrins remain inextricable. These effects were reversible upon removing the OV from the medium, indicating the presence of active phosphatases in erythrocytes. Interestingly, no reduction of the interaction between protein 4.2 and band 3 after OV treatment was observed indicating that band 3 phosphorylation does not affect this interaction. Inhibition of Src kinases (PP2) during OV treatment rescued the echinocytes appearance and fragility of erythrocytes. Furthermore, it decreased the tyrosine and serine phosphorylation of band 3. We identify LYN is the SRC-kinase family members that is activated after OV treatment. PKC inhibitors (Gö6967) also rescued the increased fragility after OV treatment, however, erythrocytes were spherocytes and tyrosine or serine phosphorylation of band 3 induced by OV treatment was not affected. This suggests that PKC elicits its effects independent from band3 and that serine phosphorylation of band 3 is independent from calcium dependent PKCs. Summary and Conclusions.These data show that LYN-induced band 3 phosphorylation weaken the interaction of the membrane with the underlying spectrin cytoskeleton thus regulating the flexibility of erythrocytes without inhibiting protein 4.2-band 3 interactions.

Infectious diseases - Bacterial and fungal

0961

THE CLINICAL SIGNIFICANCE OF RESPIRATORY VIRUSES AND COINFECTIONS IN HEMATOLOGICAL PATIENTS

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Background. The extent of respiratory virus infections in immunocompromised hematological patients is only partially elucidated. **Aims.** To determine the incidence of 12 common respiratory viruses detected in adult hematological patients admitted to hospital with respiratory symptoms, to evaluate the clinical presentations and outcome, and finally to relate and compare the findings with that obtained from current practice microbiological diagnostics. **Methods.** Throat swabs were collected and analyzed by a polymerase chain multiplex technique from 329 patients with 450 episodes of acute respiratory symptoms at two hematological departments from January 2007 through December 2007, and from December 2008 through November 2009. The following viruses were detected: influenza A, influenza B, respiratory syncytial virus, human metapneumovirus, adenovirus, rhinovirus, coronaviruses 229E, NL-63 and OC43, and parainfluenza viruses type 1, 2 and 3. Clinical data and results of laboratory and microbiological analyses were collected simultaneously. **Results.** Respiratory viruses were detected in 138 (31%) of the 450 episodes of respiratory symptoms. The most frequent viruses were rhinovirus (n=44, 32%) and influenza A (n=30, 22%). In ten of the episodes (2%), the patients had two or three viruses detected in the same sample.The most frequent diagnoses in viral-positive patients were AML (32 %) and malignant lymphoma (17 %). The number of virus-positive patients was not higher in patients with neutrophil counts below 0.5 x 10^9/l than in patients with neutrophil counts above 0.5 x 10^9/l. The frequency of viral infections was higher in patients undergoing hematological stem cell transplantation (85 of 215 patients), than in other patients (53 of 235 patients, p<0.001). There was no difference in the frequency of viral infections between autologous or allogeneic stem cell transplant patients, but significantly more viruses were detected in patients who had undergone a myeloablative allogeneic stem cell transplantation (47 of 95 patients, 49%), compared to a non-myeloablative allogeneic stem cell transplantation (29 of 88 patients, 33%, p=0.023).Coincident bacteria or fungi were detected in 49 of the 138 viral infections (36%). Bacteria considered clinically significant were detected in 19 viral infections (14%). The most frequent combinations of virus and clinically relevant bacteria were rhinovirus and H. influenzae (n=4) and rhinovirus and S. aureus (n=2).Death within 30 days of the viral sample was seen in 18 (4%) of the respiratory episodes, but the mortality frequencies did not differ between the virus-positive and virus-negative patients. Likewise, the mortality frequencies did not differ between patients with a virus-only-infection, and patients with coinfections of a virus and bacteria.**Summary.** Respiratory viruses are frequently diagnosed in hematological patients. Only in few cases was a mix of viral and bacterial etiology found. The presence of respiratory virus was not related to the degree of neutropenia and did not have an impact on the mortality. Patients who have undergone hematological stem cell transplantation were more prone to viral infections. This highlights the importance of using comprehensive diagnostic methods, including viral diagnostics, when managing acute respiratory infections in these patients.

0962

CHRONIC/RELAPSING LYMPHADENOPATHY ASSOCIATED WITH HHV-6B INFECTION: A NEW BENIGN CLINICO-PATHOLOGIC ENTITY OCCURRING IN IMMUNOCOMPETENT INDIVIDUALS

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Background. HHV-6 DNA sequences were disclosed in lymph node (LN) tissues of several patients with lymphoid malignancies, but a direct major role of HHV-6 in lymphoid malignant transformation has so far not been confirmed. In contrast, active HHV-6 infection has been associated to either infectious mononucleosis-like syndrome or acute lymphadenitis occurring in febrile

patients with systemic symptoms, or to Rosai-Dorfman disease in which viral antigens have been detected by immunohistochimical (IHC) analyses in both histiocytes and follicular dendritic cells (FDCs). **Methods.** We have retrospectively analyzed clinical and pathological data of 365 adult patients, consecutively observed at our Institution over a period of 5 years (2006-2010), because of enlarged superficial lymph nodes and subsequently undergoing lymphadenectomy. In the benign/reactive cases in which well-recognized etiologies have been excluded, an involvement of HHV-6 active infection or reactivation was investigated by molecular and immunohistochemical examinations. *Results.* Malignant disorders, namely malignant lymphoproliferative disorders or solid cancer metastases, were found in 227 cases (62%), whereas in 138 cases (38%) benign/reactive pictures were documented on lymph node examination. Among these latter cases, a well-recognized etiology was demonstrated in 84 patients (61%), while in 54 cases (39%), a well-defined non-malignant reactive/infectious cause could not be documented. Immunohistochemical analyses resulted negative for both HHV-6A and HHV-6B in 38 of these latter lymph nodes (70%). In 7 patients (13%), a scattered, scanty and aspecific positivity for HHV-6B late protein was documented in rare interfollicular plasma cells and histiocytes. Surprisingly, in 9 patients (17%), immunohistochemical analyses showed HHV-6B positive staining of FDCs, together with scattered positivity of interfollicular cells. These 9 HIV-negative adult patients (median age 42 years, range 18-76 years), with either localized or generalized LAP, were observed for a median follow-up of 38 months (range 28-166). Of note, six of them presented with recurrent LAP (one to 3 recurrences), without evolving into lymphoma. A common LN histological pattern at presentation showed florid follicular hyperplasia with concurrent mild paracortical expansion. Three cases also showed features consistent with PTGC. Constitutional symptoms were absent in all patients. The IHC reactions for both HHV-6A and HHV-6B, performed on further control cases, represented by 131 LN tissues from patients with either benign LAP induced by other known etiologies or lymphoma, were invariably negative. Serology was positive for both IgM and IgG with high avidity suggesting viral reactivation/reinfection. However, the molecular analyses failed to detect HHV-6 viremias in cell-free-serum samples of all the 9 patients with positive HHV-6B IHC staining, while positivity for HHV-6B DNA was disclosed by PCR analyses in 7 out of the 7 LN tissues studied. *Conclusions*. We show for the first time that local reactivation/infection of HHV-6B should be considered among the possible causes of chronic/relapsing benign LAP in immunocompetent individuals. IHC is the method of choice for investigating the presence of HHV-6 infection in such cases. HHV-6B may indirectly modulate and trigger the proliferation of lymphocytes, by locally affecting FDCs and LN microenvironment. FDCs may indeed be involved in presenting HHV-6B antigens to other immune cells, mainly cortical B lymphocytes.

0963

HUMAN HERPESVIRUS 6 IS A FREQUENT INFECTION IN MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION IN THE ERA OF NOVEL ANTI- MYELOMA AGENTS

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Background. Human herpesvirus 6 (HHV-6) disease, evoked by viral reactivation in the presence of immune suppression, presents with fever, skin eruption, encephalitis and pneumonia. The incidence of HHV-6 disease has not yet been studied in a large homogenous cohort of autografted patients with multiple myeloma (MM) in the era of novel agents, which are known to alter the immune system function.**Aims.** The aim of the current study was to evaluate the frequency and importance of the HHV-6 disease in consecutive patients undergoing autologous stem cell transplantation (ASCT) for MM after treatment with novel agents. **Methods.** Data on 62 consecutive MM patients treated with bortezomib-dexamethasone (VD) or thalidomide-dexamethasone (TD) induction therapy in conjunction with melphalan 200mg/m^2, who underwent ASCT between 01.2005 and 09.2010, were reviewed. According to the department protocol, during the study period, patients with an unexplained post-engraftment fever of 3 days underwent a polymerase chain reaction (PCR) screen for viral infection. Diagnosis of HHV-6 disease required a positive PCR test for this virus, with no evidence for a bacterial or fungal pathogen in blood cultures or in a chest computerized tomography scan.**Results.** Twenty one patients (34%) received TD and 41 (66%) VD. There were no statistically significant differences in patient characteristics between the 2 groups, apart from the dexamethasone dosage, which was significantly higher in patients receiving TD (640 vs 1000 mg, p=0.024). Eight patients in the TD cohort and 18 in the VD cohort experienced a post-engraftment unexplained fever, hence, underwent viral screening. Ten patients of the whole series (16%), accounting for 35% of those who had been screened, were diagnosed with HHV-6 disease. The incidence of this disease in patients treated with VD approached 19.5% (n=8) versus 9.5% (n=2) in those treated with TD. All patients recovered without sequels. **Conclusions**. HHV-6 disease is relatively common after ASCT, accounting for at least third of post-engraftment episodes of unexplained fever, with a presumably higher incidence following VD induction. Timely identification of this infection might eliminate the need for further diagnostic evaluations and contribute to the efficient management of these patients.

0964

ROMIPLOSTIM AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: RESULTS OF A PILOT STUDY

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Background. A number of studies have demonstrated that G-CSF can fasten neutrophil recovery in patients undergoing high-dose chemotherapy (HDCT) and hematopoietic stem cells transplantation (HSCT). Unfortunately, the only measure available to correct post-transplant thrombocytopenia is platelets transfusions. Recently immune thrombocytopenic purpura management has changed with introduction of thrombopoietin receptor agonists (TRA), which were highly effective and safe in such patients. To date the only two TRA are available - romiplostim and eltrombopag. It is possible that administration of TRA can significantly alter clinical course of patients undergoing HDCT. To address this question we have initiated a pilot study to assess the efficacy of TRA after HDCT with HSCT. **Patients and Methods.** Seven patients (median age, 45 years; range 21-56; 6 male and 1 female): 2 patients with non-Hodgkin's lymphoma (NHL), 2 patients Hodgkin's lymphoma (HL) and 3 patients with multiple myeloma (MM) were treated with HDCT/autologous HSCT and romiplostim support [autoHSCT+romiplostim group]. Two patients: 1 patient (20 years, male) with aplastic anemia (AA) and 1 (50 years, male) with chronic myelogenous leukemia (CML) were treated with allogeneic HDCT/HSCT and romiplostim support [alloHSCT+romiplostim group]. Romiplostim was given subcutaneously at a dose of 250 μg on day of HSC infusion. Twelve patients (median age, 39 years; range 19-64; 6 male and 6 female) with NHL (n=6), HL (n=5) and MM (n=1) were assessed as a control group (without romiplostim support). The endpoints of the study were time of platelets recovery (TPR), duration of thrombocytopenia (DT), and length of hospital stay (LHS). Statistical analysis was conducted with nonparametric statistical methods (Mann-WhitheyU test for continuous variables and Fisher exact and Chi-square tests for categorical variables).**Results.** The DT grade 3 was 10.7 days in the autoHSCT+romiplostim group in comparison with 19.0 days in the control group (*p=0.044*). DT grade 3 in alloHSCT+romiplostim group was 57 days for AA patient and 29 days for CML patient. DT grade 4 was 6.9 and 13.8 days in average groups, respectively *(p=0.259)*. DT grade 4 was 54 days for AA and 3 days for CML patients. TPR grade 3 was 13.9 days for autoHSCT+romiplostim group and 22.2 days for the control group (*p=0.044*). TPR grade 3 was 48 days for AA patient and 35 days for CML patient (alloHSCT+romiplostim group). TPR grade 4 was 13.0 and 18.7 days according to study groups (*p=0.035*). TPR grade 4 was 45 days for AA patient and 11 days for CML patient (alloHSCT+romiplostim group). LHS was 27.9 in the autoHSCT+romiplostim group in comparison with 36.7 days in the control group (*p=0.021*). LHS was 60 for AA patient and 45 days for CML patient (alloHSCT+romiplostim group). **Discussion.** The duration of thrombocytopenia, times of platelet recovery and the length of hospital stay are shortened when romiplostim is administered after HDCT. This tendency confirms the rationale for use of romiplostim which can improve patients' outcome in setting of HDCT and HSCT. Further studies are needed to evaluate the efficacy and safety of romiplostim support as a part of post-transplant care for auto- and allograft recipients.

0965

CAN JC VIRUS BE PREEMPTIVELY DETECTED PRIOR TO THE DEVELOPMENT OF MULTIFOCAL LEUKOENCEPHALOPATHY IN PATIENTS UNDERGOING ALLOGNEIC STEM CELL TRANSPLANTATION?

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Background. Recent studies demonstrated an increased incidence of JC virus (JCV)-related progressive multifocal leukoencephalopathy (PML) in immunocompromised patients. PML, mostly fatal, has been reported in transplanted patients, including those undergoing allogeneic stem cell transplantation (alloSCT). The expanding application of alloSCTs nowadays, reflecting the growing availability of matched unrelated donors, is expected to increase the incidence of this complication.Therapeutic options in PML are extremely limited and usually unsuccessful, resulting in a rapid neurological progression and

death. However, immune restoration, whenever possible, is claimed to be highly important for PML treatment.**Aims.** The aims of the current study were to determine the natural course of JCV reactivation and the correlation between serum JCV levels and immunosuppression and to explore the possibility for preemptive detection of JC reactivation prior to PML clinical development. **Methods.** The study was approved by the Rambam IRB (approval # RMB 0097-12). Two patients (out of 116 allografted at the Rambam Medical Center over the last 2 years), were diagnosed with PML based on positive PCR test for JCV in blood and cerebrospinal fluid (CSF), in the presence of a typical brain MRI scan. Data, focusing on immunosuppressive therapy administered, clinical presentation and course of PML, were recorded. DNA samples of both patients, originally taken for quantitative CMV-PCR analysis, were evaluated by JCV-PCR during the transplant period. JCV was detected by quantitative RT0-PCR of whole blood. **Results.** The two patients were diagnosed with PML 188 days or 195 days after alloSCT (Table 1). Reassessment of stored blood samples revealed JCV reactivation in both patients, which occurred 126 or 111 days prior to appearance of neurological symptoms and PML diagnosis. These two patients were heavily pretreated before alloSCT. Moreover, their post-alloSCT period was complicated with chronic relapsing GvHD and repeated severe infections. They received immunosuppressive drugs, including steroids. The patients were JCV-negative during and early after the alloSCT, but became persistently positive (on days 62 or 84 post-transplant) long before developing PML (table 1). Remarkably, their JCV blood levels tended to correlate with steroid dosage.

Days from SCT	Patient 1		Patient 2	
	JC virus#	Steroid dosage†	JC virus#	Steroid dosage†
62	8000	0	0	0
84	8750	0	2200	0
97	912,000	40	5000	0
132	110,000	20	NA	40
153	620,000	40	8000	40
173	500,000	40	3500	20
188	**2600000* PML**	40	3700	20
195	2,200,000	20-10	**4500*PML**	20
209	1,350,000	60	7000	10
219	25,000,000	60	NA	10
226	19,000,000	60	3000	20
246	>100,000,000	60	NA	20

Quantitative PCR in copies/ml; † Steroids dosage in mg adjusted to GvHD severity;
* Clinical PML with JC in CSF and MRI findings

Conclusions. The findings of the current study suggest that preemptive JCV monitoring and management should be considered in heavily pretreated alloSCT patients. The revealed correlation between steroid doses and JCV may imply that early detection of the virus reactivation and reduction in immunosuppression could ultimately lead to prevention of clinical PML. Prospective studies are warranted for the elucidation of these issues.

0966

EFFECT OF A SINGLE DOSE OF ANTI D (WIN RHO*) IN REDUCING HEMORRHAGIC COMPLICATIONS IN PATIENTS HAVING SEVERE THROMBOCYTOPENIA SECONDARY TO DENGUE VIRUS INFECTION

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Background. Dengue is an important cause of acquired thrombocytopenia in tropical counties. Although self limiting, it may result in life threatening hemorrhagic complications. **Aims.** In this study our aim was to evaluate the efficacy of a single dose of Anti -D in reducing hemorrhagic complications due to severe thrombocytopenia (<20,000) in dengue virus infection. **Methods.** An open label, investigator initiated , randomized interventional study was conducted at our centre. Patients presenting with fever and thrombocytopenia were screened and evaluated for enrolment. 30 patients with diagnosed dengue haemorrhagic fever with severe thrombocytopenia (platelet count ≤20,000/m^m3) were enrolled. They were divided into two groups. The intervention group received injection Anti - D , 50 µg/kg (250IU/kg) along with the standard care while the control group received standard care only. Both the groups reeived platelet transfusions when indicated. The base line parameters were recorded Platelet count at presentation, the grade of bleeding , improvement in the bleeding grades, the volume of the platelet transfused and the duration of hospital stay were compared in both the groups. Any serious adverse event was noted and reported Continuous data was expressed as Mean (S.D.) and / or Median (range). Categorical data was expressed as % 95% Confidence interval was calculated for all the parameters For intergroup comparisons Paired T-test was used and for intragroup comparison Wilcoxon sum rank test was used. For categorical data, Chi Square test or Fischer Exact test was used p value of < 0.05 was considered significant. **Results**. A total of 30 patients were included in the study. Baseline parameters were comparable. 73.3% patients were in the age group of 20 - 50 years, with a male predominance; (93.3%) The mean duration of symptoms was 4.2(±1.14) days (intervention group) as compared to 5.53 (±1.92) days (control group). The baseline platelet count of the intervention group 12,266 (±3614.78) was low as compared to the control group platelet count 14,333(±3579.04). 6 (40 %) patients in the control group and 5 (33.3 %) in the intervention group who had grade 1 bleed at presentation whereas 7 (46.7%) patients in the control group and 5 (33.3 %) in intervention group had grade 2 bleeding at presentation Although there was no deterioration in the bleeding grades in either group but the improvement in the bleeding grades was rapid in the intervention group of the patients as compared to the control group. The requirement of the platelet concentrate infusion in the control group was significantly higher 342.3 ml (±192.61) as compared to the intervention group requiring only 187.3 ml (±79.16) The mean duration of hospital stay was comparable in both groups, 5.8 days (control) vs 5.78(intervention group). There were no serious adverse events noted. **Conclusions**. Bleeding in patients having severe thrombocytopenia (20,000/mm3) secondary to Dengue Virus Infection was rapidly and safely reversed by administration of a single dose of 50 µg/kg (250IU/kg) Anti D intravenously.

0967

HIGH DOSE PROPHYLACTIC ACICLOVIR IMPROVES OUTCOMES IN ADULT UNRELATED DONOR STEM CELL TRANSPLANTATION

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BACKGROUND. Cytomegalovirus (CMV) disease remains an important cause of morbidity and mortality following allogeneic stem cell transplantation (SCT) particularly in recipients of unrelated donor transplants. Pre-emptive ganciclovir (GCV) treatment, although effective, is associated with significant toxicity. The role of prophylactic strategies remains unclear and considerable uncertainty still surrounds the benefit of high dose aciclovir (ACV) in reducing the risk of CMV infection and death after allogeneic SCT.AIMSWe wished to compare the impact of two different ACV prophylaxis strategies on CMV-related mortality and transplant outcome in adult unrelated donor stem cell transplant recipients.METHODSDemographic and clinical data were collected on all patients undergoing matched unrelated SCT between January 2002 and December 2010 at risk of reactivating CMV (defined as CMV seropositive patient or donor prior to SCT). 144 adults with a haematological malignancy underwent transplantation from a matched (7/8 or 8/8 match at HLA-A,B, C or DRb1) donor. 53 (37%) patients were transplanted using a myeloablative conditioning regimen (Cy/TBI) and 91 (63%) using a fludarabine-based reduced-intensity regimen. Alemtuzumab (10mg/day x 5 days) was administered to all patients on days -5 to -1. Between January 2002 and July 2004, 24 patients at risk of CMV infection received "low dose ACV"(LDACV) (200mg PO q.d.s) until day +35. Between August 2004 and December 2010, 120 patients received a "high dose ACV"(HDACV) schedule (500 mg/m^2 IV t.d.s followed by 800 mg qds po until day+100). CMV viral loads were measured at weekly intervals until day +100 and patients who developed CMV viraemia immediately commenced therapeutic doses of GCV. The method of Kaplan and Meier was used for comparison between treatment groups. Survival rates are reported with 95% confidence intervals at 100 days and 6 months post transplant with no patients lost to follow up.RESULTSAn improvement in overall survival (OS) was observed at 100 days with 87% OS in the HDACV group (95% CI: 79% to 92%) compared with 71% OS in LDACV (95% CI: 48% to 85%) (Figure 1). CMV-specific mortality at 100 days was reduced in the HDACV group (CMV survival rate: 0.99, 95% CI: 0.94 to 1.00) compared with the LDACV group (CMV survival rate: 0.82, 95% CI: 0.59 to 0.93). The reduction in CMV-specific mortality was still apparent at six months; HDACV CMV survival rate of 83% (95% CI: 0.75 to 0.89) vs LDACV CMV survival rate of 74% (95% CI: 0.51 to 0.87). The incidence of CMV disease was also lower at day 100 post transplant (OR=0.24, 95% CI: 0.07 to 0.95) and at 6 months (OR=0.27, 95% CI: 0.08 to 1.04). Overall survival rate at 2 years was 58% in the HDACV group (95% CI: 49% to 67%) compared with 38% in the LDACV group (95% CI: 19% to 56%).CONCLUSIONSOur data indicate that high dose ACV may have the capacity to reduce early CMV-related mortality in recipients of unrelated donor transplants. The

optimal dose of prophylactic ACV in patients undergoing alternative donor SCT requires examination in prospective randomized trials.

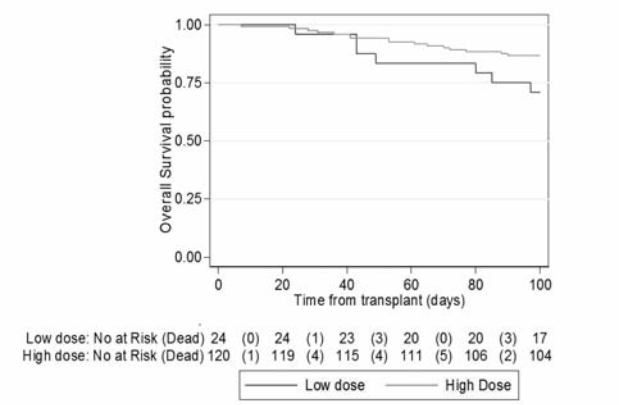

Figure 1. Kaplan Meier survival curve up to 100 days post transplant

0968

SERUM FREE LIGHT CHAINS IN PATIENTS WITH HIV: THEIR ASSOCIATION WITH MARKERS OF DISEASE STAGE AND SEVERITY, AND THE EFFECT OF ANTIRETROVIRAL THERAPY

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Background. Monoclonal serum free light chain (FLC) measurements are used to follow up and manage patients with monoclonal gammopathies, and abnormal serum free light chain (FLC) ratios have been associated with risk of progression in certain diseases. B-cell dysfunction has been well-described in HIV infection and B-cell abnormalities are associated with disorders leading to monoclonal gammopathies and abnormal FLC levels in other studies. However, to date, only one publication describes FLC levels in HIV infection. **Aims.** To determine whether the B-cell dysfunction in HIV leads to abnormal FLC levels and to correlate these levels with recognized markers of disease in HIV. **Methods.** We determined serum albumin, IgG, $CD4^+$ counts, viral loads and kappa (k) and lambda (λ) FLC levels on 369 HIV positive subjects. The k and λ FLC results were used to calculate a FLC ratio. These results were then correlated with $CD4^+$ counts, viral loads, IgG, albumin, stage of disease and the use of antiretroviral treatment (ART).Results. Our study population consisted of 66% females with a mean age for the whole population of 37.7 years (95%CI = 36.8 - 38.6). Most were black Africans (66%) followed by 26% of mixed ethnicity, with the remaining 8% being either Caucasian, of unknown race or another race. Eighty-nine percent were on ART. The k FLC values ranged from 5.59-387.0 mg/l (median 19.6 mg/l) and the λ FLC ranged from 9.28-286 mg/l (median 22.3 mg/l). Both k and λ FLC concentrations positively correlated with viral load ($p<0.05$) and IgG ($p<0.05$) and negatively correlated with $CD4^+$ counts ($p<0.05$) and albumin levels ($p<0.05$). Lambda FLC levels significantly correlated with stage of disease ($p<0.01$). Of all disease markers evaluated, the FLC ratio only correlated with IgG levels ($p<0.05$). A significant correlation was found between k and λ FLC and patients taking ART ($p<0.01$), with those on ART having lower FLC levels. However, the FLC ratio was not significantly affected by ART use ($p=0.98$). **Conclusions.** We demonstrated that FLC levels were significantly correlated with markers of HIV disease severity in our population. The fact that individual FLC levels but not the FLC ratio correlated with these markers may be due to the well-described polyclonal hypergammaglobulinemia that is associated with B cell dysfunction in HIV-infection. We postulate that individual FLC measurements may be of value in the follow up and monitoring of response to therapies in HIV positive patients.Acknowledgement: The Binding Site for supplying the FLC kits to perform this study and for providing ongoing scientific support.

0969

ANTHRACYCLINES THERAPY INDUCES CHANGES IN ECG IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML).

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Background. Anthracyclines are widely used in AML therapy. Cardiotoxicity is a serious complication of anthracyclines treatment especially in patients with cardiovascular comorbidities.Variety methods have been used for evaluation of early cardiotoxicity in patients during and after anthracyclines administration. The R wave in lead aVL reflects left ventricle (LV) mass. The R wave changes may be associated with higher risk of cardiovascular disease.AimThe aim of this study was to evaluate the voltage of wave R and QRS in lead aVL and its significance as an indicator of early cardiotoxicity in patients with AML after anthracyclines-based chemotherapy.Materials and methods50 patients with newly diagnosed AML were included in the study (20 females and 30 males). The median age of patients was 47 years (range:18-65 years). 12 (24%) patients had hypertension (WHO 1 and 2) and 4 patients (8%) had type II diabetes mellitus.All patients were treated following the standard chemotherapy protocol based on daunorubicin in dose 60 mg/m^2 and conventional dose of cytarabine arabinoside (Ara-C). 12-lead ECG records with a paper speed of 50 mm/s were performed in all patients before chemotherapy and after first cycle with anthracyclines. ECG voltages were measured manually. In patients with hypertension and diabetes mellitus ECHO was performed. We evaluated ECG parameters as follows: R-wave voltage and total QRS duration, PQ interval and presence of repolarization changes in lead aVL.Statistical analysis was performed by means of Mann-Whitney's U-test using 'STATISTICA 8,0 software 'and $p<0,05$ indicated a significant difference.ResultsThe voltage of wave R in lead aVL after anthracyclines-based chemotherapy was significantly higher in comparison with values before treatment (0,6±0,13 mV vs 0,4±0,11 mV; $p<0,01$). The difference was even higher in patients with hypertension and diabetes mellitus. The total QRS voltage decreased significantly after chemotherapy (4,15±1,12 mV vs 4,59±1,56; $p<0,01$). Repolarization changes were observed in 5 patients (10%). We found no significant correlation between PQ interval at baseline and after chemotherapy. The results are shown in table 1.ConclusionOur results show that the value of voltage of the wave R in lead aVL could be the early indicator of anthracyclines-based cardiotoxicity before other changes in ECG and ECHO especially in patients with cardiovascular diseases .This observation should be validated by larger study.

Table 1: ECG measurements before and after anthracyclines chemotherapy in AML patients.

aVL mV	Before chemotherapy N=50	After chemotherapy N=50	P-value
R	0,4±0,11	0,6±0,13	<0,01
QRS	4,59±1,56	4,15±1,12	<0,01
PQ	0,15±1,23	0,16±1,54	0,08

N=number of patients

0970

INTERFERON-GAMMA MRNA EXPRESSION CAN PREDICT VIROLOGIC RESPONSE TO HEPATITIS C VIRUS THERAPY IN PATIENTS WITH HCV GENOTYPE 4

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Background and Aims. Ribavirin & peginterferon is the most effective treatment of chronic hepatitis C but has unpleasant side effects and high costs. A large proportion of patients do not respond to therapy for reasons that are unclear. We hypothesized that pretreatment gamma interferon gene expression level could be used to predict treatment outcome (responders and non-responders) in Egyptian HCV patients. **Subjects.** This study involved 29 HCV subjects; they were classified after the 24 weeks of a treatment regime (Ribavirin & peginterferon) into two groups (16 patients with non-detectable HCV classified as responders & 13 patients who had detectable HCV classified as non-responders). **Methods**. Baseline gamma interferon (IFN gamma) gene expression was measured by real time-polymerase chain reaction (RT-PCR) methods using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping gene. **Results**. Levels of IFN-gamma expression were higher in the non-responder group compared with the responder group ($p<0.05$). There was no correlation between IFN-gamma expression levels, stage of fibrosis or viral

load level. **Conclusions.** An interferon gamma gene expression cutoff level of 0.54 at baseline (before starting the treatment regime) can discriminate patients with response from patients with failure of response. These data may be of use in predicting clinical responses to treatment

0971

LARGE UNSTAINED CELLS ON ROUTINE HEMATOLOGY ANALYZER CORRELATE WITH IMMUNE ACTIVATION LEVELS IN ASYMPTOMATIC TREATMENT-NAÏVE HIV INFECTION

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Background. HIV infection is characterized by ongoing immune activation and inflammation which drive the loss of CD4 T cells and progression to AIDS. Furthermore, despite adequate control of viral loads, patients are at increased risk of inflammatory-associated complications such as cardiovascular disease and cancer. The expression of the activation marker CD38 on CD8 T cells is a valuable measurement of immune activation levels in HIV-infection and has been shown to predict for poor prognosis in untreated patients and for reduced recovery of CD4 counts on antiretroviral therapy (ART). However, CD38 expression on CD8 T cells is measured by Flow Cytometry which may not be readily available in resource-limited areas. HIV continues to devastate Sub-Saharan Africa where the disease burden remains highest. Cost-effective screening measures to determine levels of immune activation may impact on management strategies in resource-limited settings. A full blood count is regularly requested in the work-up of these patients and may add value where Flow Cytometry is not readily available.aim:Our aim was to develop a method to assess immune activation and HIV disease progression, in resource-limited settings. The proposed method would have to be cost-effective and easy to perform on the equipment available in these settings.methods:Patients were recruited from a primary health clinic inCape Town,South Africa. The 80 cases were HIV-infected adults who were ART-naïve and clinically well, with 50 uninfected age- and sex- matched controls. Blood from these patients was used to measure: the CD38 expression on CD8 cells (%CD38onCD8), the percentage of large unstained cells (%LUCs), the CD4 count, the viral load and the total lymphocyte count. The %CD38onCD8 and CD4 counts were analyzed using Flow Cytometry; the viral load was quantified using Polymerase Chain Reaction (PCR)); and the total lymphocyte count and %LUCs were performed on a standard Hematology laboratory analyzer, the Siemens ADVIA 2120 system. The %LUCs usually include: virally activated lymphocytes, plasma cells, hairy cells, pediatric lymphocytes and peroxidase negative blasts. The %LUCs, %CD38onCD8, CD4 count, total lymphocyte count, and viral load were tested for statistical significance, and were then correlated against %LUCs. results:There were a statistically significant differences between the case and control groups, for the %LUCs (mean 2.5±0.9 vs 2.0±0.9; p=0.001); %CD38onCD8 (mean 57.8±19.7 vs 40.3±16.4; p=0.000) total lymphocyte counts (mean 1.7±0.5 vs 2.1±0.8; p=0.01); and CD4 counts (mean 405±177 vs 952±332; p=0.000). There was also a statistically significant positive correlation between %LUCs and %CD38onCD8 (r=0.3241; p=0.000) and a significant inverse correlation between %LUCs and CD4 counts (r= -0.248; p=0.006). There was no correlation observed between the %LUCs and the total lymphocyte count (p=0.570) or the viral load (p=0.208).conclusion:Our study found statistically significant correlations between %LUCs and both %CD38on8 and the CD4 count. The %LUC test is relatively cost-effective and easy to perform as part of the differential count, which is often requested with a full blood count. This suggests that large unstained cells may serve as a useful prognostic marker for HIV disease progression, in a resource-limited setting.

0972

SAFETY AND EFFICACY OF AN EDUCATIONAL PROGRAM IN REDUCING THROMBOTIC AND INFECTIVE COMPLICATIONS OF PERIPHERALLY INSERTED CENTRAL CATHETERS (PICCS) IN HAEMATOLOGICAL PATIENTS

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Purpose. Patients with haematological disorders frequently require the insertion of medium or long-term central venous catheters (CVCs) for stem-cell transplantation, the administration of chemotherapy, or transfusion of blood products. Although peripherally inserted central catheters (PICCs) have been in use for many years, little data exist on their use in patients receiving intensive chemotherapy. **Methods.** Evidence-based interventions were implemented in our department in December 2010, and include:1.An high level nurse education program for correct practices and prevention of catheter-associated complications. was developed for PICC nursing team;2) The use of ultrasound guide for the insertion of the tip of PICCs, thanks to a special operator training; 3) Bedside placement and confirmed PICC tip placement by chest radiography after removal of the guidewire and before the securing of the catheter; 4) Maintenance of maximum sterile barrier precautions during PICC insertion and aftercare; 5) chlorhexidine preparation, replace 10% povidone iodine for skin antisepsis; 6) adoption of PICC patient nurse archive, including the information of weekly PICC line review at our department for each patient.Here, we carried out a clinical investigation to determine the efficacy of these interventions in reducing the rate of PICC-related complications (thrombotic events, exit site infection and other complications requiring early removal of PICCs) and to compare PICCs-specific complications (CR-BSI) with a cohort population defined as each consecutive CVC non tunneled inserted over a 6-month period (May-November 2009), and before these evidence-based interventions. **Results.** Ninety-fivePICCs were in place for a total of 7,295 PICC days (range, 1-331 days; mean, 76,7 days), and fifty-one CVCs were inserted before these interventions (range, 3-577 days; mean, 176,2 days). Sixty-six PICCs were inserted during severe thrombocytopenia (platelets < 50 x 10(9)/L), and 70 during severe neutropenia (neutrophils < 0.5 x 10(9)/L). The majority of the patients were affected by leukaemia, and PICCs were inserted to ensure adequate access throughout chemotherapy. Other mechanical complications occurred in 11 catheters, and were accidental dislodgement (4), catheter break (3), catheter inadequate (4). Compared with CVCs group, the PICCs group was associated with a lower incidence of CRBSI complication rate during neutropenia (1,05% vs 41,17%, 0,14 vs 3,67 per 1,000 CVC days) [odds ratio (OR) 0,051; relative odds reduction (ROR) 0,98]. The rate of thrombotic complications was lower in PICCs group (0,27 per 1,000 CVC days, vs 0,70 per 1,000 CVC days) [odds ratio (OR) 0,252; relative odds reduction (ROR) 0,747]. **Conclusions.** Our results indicate that a training and competence assessment program is effective in reducing the main complications PICCs-related in haematological setting.

Tab 1. Catheter outcomes, by device

	n=95 PICCs		n=51 CVCs		
	Number	Percentage (%)	Number	Percentage (%)	
Male	48	50.5%	30	58,8%	
Female	47	49,40%	21	41,2%	
Mediane Age	Range 17-82 years	49.1	Range 23-78 years	50,5	
Type of catheter:					
Silicon rubber	6	6.3%	CVC ST (short term)	35	68,63%
Polyurethane	3	3.1%	CVC LT (Long term)	16	31,37%
Polyurethane power injectable	86	90.5%			
Definite CRBSI					
	% infection	Per 1000 CVC days	range (days)	Means (days)	OR
PICCs (n.95)	1,05	0,14	1-331	76,79	**0.0151**
CVCs (n.51)	41,17	3,67	3-577	176,2	
Thrombotic complications					
PICCs	2,11	0,27	1-331	76,79	**0,25**
CVCs	7,84	0,70	3-577	176,2	

Stem cell transplantation - Clinical 2

0973

MINIMAL RESIDUAL DISEASE AS A PREDICTIVE FACTOR FOR ADVERSE OUTCOME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Allogeneic hematopoietic stem cell transplantation (allo-SCT) is potentially curative for patients with high-risk leukemia, but disease recurrence remains the leading cause of treatment failure. Our **aim** was to determine the impact of minimal residual disease (MRD) by any technique in adult patients with acute myeloid leukemia (AML) in morphologic first (CR1) and second complete remission (CR2) undergoing allo-SCT in predicting adverse outcome. **Materials and methods.** Our study group consisted of AML patients in first and second complete remission treated in the Hematologic Malignancies/Stem Cell Transplant Unit at the UCLA, from January 2000 through January 2010 using allogeneic bone marrow, blood-derived and cord blood stem cells from histocompatibile related and unrelated donors. For the MRD assessment we used multiparametric flow cytometry, cytogenetics and fluorescent in situ hybridization. Any level of residual disease was considered MRD positive. **Results.** 59 patients were eligible for the study of 160 patients transplanted over ten years; 19 patients (32.2%) were identified as MRD positive. Patients with MRD had a consistently worse outcome over those without MRD, with 3-years leukemia-free survival (LFS) of 15.8% vs. 62.4 % and overall survival (OS) of 17.5% vs. 62.3%. Relapse rate was significantly higher in MRD-positive patients; 3 years relapse rate in MRD-positive patients was 57.9% vs. 15.1% in MRD-negative patients. Detection of MRD in complete remission was associated with increased overall mortality (HR=3.3; 95% CI: 1.45-7.57; p=0.0044) and relapse (HR=5.26; 95%CI: 2.0-14.0; p=0.001), even after controlling for other risk factors. There was no statistically significant difference in the MRD techniques employed in predicting adverse outcomes. **Conclusions.** Our study showed that for patients in morphologic complete remission the presence of MRD predicts for significantly increased risk of relapse and reduced LFS and OS. Presence of MRD in pre allo-SCT AML patients defines a high risk group of patients. Further therapeutic possibilities in these patients, such as post-transplant donor lymphocyte infusions (DLI), alternative high dose conditioning regimens, adjuvant treatments, or other early therapeutic intervention should be considered.

0974

COINFUSION OF HAPLO-IDENTICAL DONOR STEM CELLS WITH AN (UN)RELATED CORD TRANSPLANT PROVED TO BE SUCCESSFUL IN A VERY HIGH RISK GROUP OF PATIENTS

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Background. Combining haplodonor stem cells with a full graft cord blood unit has been proposed as a cell support mechanism which can make single cord blood available as a donor source for allogenic HSCT to a larger proportion of patients: e.g. patients with only CB donors available with low NC counts or active infection.MethodsSince 2009 we have a CB + haplo protocol for this groups of patients, with either active ongoing infection or a high probability of graft failure. Patients (with any indication) with active infection (e.g. fungal) as well as patients with only one CB-unit available below the lower acceptable NC number cut-off were offered CB + Haplo grafts. Conditioning: 1st HSCT myeloablative busulfan with therapeutic drug monitoring + fludarabine and ATG; 2nd HSCT reduced intensity with Treosulfan, fludarabine and campath The haplografts were CD34+ selected (except 1: CD3/CD19 depleted). After infusion of the CB, 5 milj/kg Cd34+ haplo-cells were infused. G-CSF was given from day +7. All patients received GvHD profylaxis with CsA and pred 1 mg/kg.Results.11 patients (10 children, 1 adult; 10 with active infection, 1 low cell count CBU) were transplanted with haplo-cord transplantation. Median age was 12,4 yr (0.25-42,2 yr). 8 of them had non-malignant disease (6 immunodeficiencies, 1 Osteopetrosis, 1 AA), 3 had a malignant indication. For 2 patients it was their second transplant. All patients but one engrafted at a median time of 12 days post HSCT (9-15). Median thrombocyte engraftment (TBC50) was 29 days (14-300). EFS was 27 % after a median follow-up of 324 days (14-1387). Incidence of GVHD gr. 2-4 was 18%. The non-relapse mortality was 2/11 (day 14 and day 24 respectively) The initial chimerism at 1 month post SCT showed > 80% haplo chimerism in most patients. However, all reach full donor *cord blood* chimerism (>95%) by a median of 121 days (28-925 days) post SCT.ConclusionCoinfusion of a CD34 selected haplo graft with (unrelated) CB transplantation is a safe and effective option for a group of very high risk patients (including patients with higher non-engraftment risks) Haplo-support leads to early haplo-engraftment, switching to full CB donor chimerism within 4 months, allowing a normal immune recovery and repertoire. A remarkably low incidence of GVHD was observed for this high risk population

0975

IS THE USE OF 9/10 HLA UNRELATED DONORS STILL ACCEPTABLE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCIES?

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Introduction. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents the only potential to cure wide types of hematological diseases. The use of 10/10 HLA matched unrelated transplants has been used as a main alternative and with its unavailability, when available, a 9/10 HLA mismatched unrelated transplant has been used. **Aims.** To evaluate the outcomes of allo-HSCT from 9/10 HLA mismatched unrelated donors compared to those from 10/10 HLA identical unrelated donors and siblings; and to define which category of patients can benefit the more in each alternative. **Methods.** We have retrospectively studied the outcome of 213 patients who received allo-HSCT for different hematological malignancies, 121 (57%) from HLA identical siblings, 63 (29%) from 10/10 HLA identical unrelated donors and 29 (14%) from 9/10 HLA mismatched unrelated donors treated during the same period of time between 2006 and 2011 at our institution. The different characteristics are detailed in Table 1.

TABLE 1 : Patients characteristics and outcomes

	HLA identical siblings group N=121	10/10 HLA unrelated group N=63	9/10 HLA unrelated group N=29	p-value
PATIENTS DESCRIPTION				
Gender				
Male	69 (62%)	37 (59%)	18 (62%)	NS
Female	52 (38%)	26 (41%)	11 (38%	NS
Age				
Median, years	45 (18-66)	44 (20-64)	38 (19-61)	NS
Disease				
Myeloid leukemia	71 (59%)	23 (37%)	16 (55%)	0,01
AML / MDS	54 / 7	15 / 0	13 / 2	
CML / MPS	3 / 7	2 / 6	1 / 0	
Lymphoid leukemia	50 (41%)	40 (63%)	13 (45%)	0,01
ALL / MM	18 / 13	20 / 15	7 / 2	
Lymphoma / CLL	14 / 5	5 / 0	4 / 0	
Disease status				NS
CR1/CP1	40 (33%)	23 (37%)	9 (31%)	
CR2/CP2	26 (21%)	7 (11%)	7 (24%)	
<CR2/CP2	55 (45%)	33 (52%)	13 (45%)	
Conditioning				NS
Full-intensity	64 (53%)	32 (51%)	16 (55%)	
Reduced-intensity	57 (47%)	31 (49%)	13 (45%)	
Cell source				
PBSC	55 (45%)	28 (44%)	13 (45%)	
BM	66 (55%)	35 (56%)	16 (55%)	
Sex-mismatching				0,03
Fd-Mr	30 (25%)	9 (14%)	8 (28%)	
Md-Fr	22 (18%)	19 (30%)	9 (31%)	
CMV-mismatching				0,07
D- R+	25 (21%)	13 (21%)	8 (28%)	
D+ R-	16 (13%)	14 (22%)	6 (21%)	
ABO-mactching				
major	22 (18%)	21 (33%)	6 (21%)	<0,0001
minor	7 (9%)	20 (32%)	8 (28%)	
Interval diag-HSCT	16 months (2-219)	14 months (4-136)	9 months (4-87)	NS
Median FU	18 months (0-60)	10 months (0-48)	8 months (0-54)	
RESULTS				
Egraftment	120 (99%)	60 (95%)	26 (90%)	0,03
CI aGVHD at 3m.	27% (23-32)	20% (15-26)	32% (23-41)	NS
CI cGVHD at 12m.				
Limited	14% (11-18)	11% (7-15)	4% (0-8)	NS
Extensive	17% (14-21)	9% (5-13)	21% (13-30)	NS
Median OS	60 months (31-NR)	18 months (11-NR)	10 months (5-21)	<0,001
CI relapse at 12m.	17% (14-20)	15% (9-20)	26% (17-35)	NS
CI TRM at 12m.	12% (9-15)	33% (27-39)	45% (35-55)	<0,0001

Results. After HSCT, engraftment was significantly lower in the 9/10 HLA group (90%) than in the 10/10 HLA group (95%) than in the sibling group (99%), (p=0.03); the cumulative incidence of acute GVHD≥2at 3months was 32% (23-41), 20% (15-26) and 27% (23-32) respectively; the cumulative incidence of extensive chronic GVHD at one year was 21% (13-30), 9% (5-13) and 17% (14-21) for the 3 groups respectively. After a median follow-up of 8 months (0-54) in the 9/10 HLA group, 10 months (0-60) in the 10/10 HLA group and 18 months in the siblings group, the median overall survival (OS) was 10 months (5-21), 18 months (11-NR) and 60 months (31-NR) respectively with a 2-years probability of 19% (8-44), 43% (31-59) and 63% (54-74) respectively (Figure). There

was a higher but not significant relapse incidence at one year in the 9/10 HLA group compared to other groups while the transplant related mortality was significantly higher with a cumulative incidenceat 1year of 45% (35-55), (p<0.001) (Table1-results). In multivariate analysis, OS was negatively affected by unrelated donors [9/10 HR=5 (2.7-10), p=0.0001; 10/10 HR=2 (1.2-4), p=0.01], female donors [HR=2 (1.4-4), p=0.03] and disease status < CR1 or <0.001; 10/10 HR=4 (1.2-10), p=0.03], female donors [HR=3 (1.2-7); p=0.01] and ABO minor incompatibility [HR=2.5 (1.2-5), p=0.01]. The funnel plot showing the adjusted TRM according to all covariates and comparing to the global population death rate, shows that the 9/10 HLA group has the worse TRM independently of any other factor.**Conclusion**We showed that allo-HSCT from 9/10 HLA mismatched unrelated donors have a significantly worse OS than those from matched unrelated donors and siblings; this was mainly due to an increased TRM in this group. Patients in first CR or CP could benefit the more from matched or 9/10 unrelated allo-HSCT while the use of transplants from 9/10 HLA unrelated donors in patients not in CR should be limited to clinical trials.

0976

FAVORABLE OUTCOME OF REDUCED-INTENSITY STEM CELL TRANSPLANTATION FOR ADULT T-CELL LEUKEMIA

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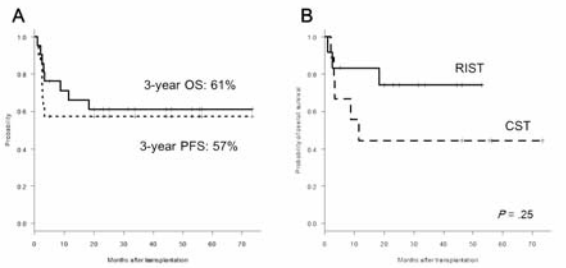
Background. Adult T-cell leukemia (ATL) endemic in southwestern Japan is an HTLV-I-induced mature T-cell neoplasm. It has a dismal prognosis when treated with conventional chemotherapy, mainly because ATL cells overexpress multidrug-resistance genes. Allogeneic hematopoietic stem cell transplantation (allo-SCT) has been reported to improve the prognosis. However, it is also known that allo-SCT shows high treatment-related mortality (TRM) because of disease characteristics of severely deteriorated cellular immunity. **Aims**. We evaluated the outcome of reduced-intensity stem cell transplantation (RIST), compared to conventional stem cell transplantation (CST) for ATL patients.



Methods. We retrospectively analyzed consecutive 21 patients with aggressive ATL (acute type, 17; lymphoma type, 4) who underwent allo-SCT between 2001 and 2011 in our institute. The median age at transplantation was 55 years (43-65). All patients received induction chemotherapy with Japan Clinical Oncology Group regimen (VCAP-AMP-VECP) or biweekly CHOP and disease status at transplantation was 3 CR, 11 PR, 4 SD and 3 PD. The median time from diagnosis to transplantation was 7 months (4-14). Nine patients received a conventional myeloablative conditioning with CY/TBI-based (n=8) or BU/CY-based (n=1) regimen and 12 patients received a reduced-intensity regimen (fludarabine 25 mg/m^2 on days -6 to -2 + melphalan 40 mg/m^2 on days -4 and -3 ± TBI 4 Gy on day -1). The age of patients who received RIST was significantly older than that of CST (median 59 vs 49, P < .001). The other parameters were balanced between the two groups. Stem cell source was bone marrow (n=17) and peripheral blood (n=4). Graft-versus-host-disease (GVHD) prophylaxis consisted of cyclosporine plus short-term methotrexate (sMTX) for HLA-matched sibling donor recipients and tacrolimus plus sMTX for the others. *Results.* The estimated 3-year overall survival (OS) and progression-free survival (PFS) rates for all patients were 61% and 57%, respectively (Figure 1A). At the median follow-up duration of 27 months (1-73), 13 patients were alive. The cumulative incidences of disease-associated mortality and TRM at 3 years were 20% and 19%, respectively. Importantly, after 1 year post-transplantation, the curve of PFS reached plateau phase. Therefore, allo-SCT could be a curable therapy for ATL. It is notable that RIST showed excellent OS. Although not significant, 3-year OS for RIST and CST were 74% and 44% (P = .25), respectively (Figure 1B). TRM of RIST trended to be low compared with that of CST (17% vs 22%, P = .80). In addition, chronic GVHD was observed in 83% of the evaluable 12 patients. It is suggested that both improvement of TRM and the induction of graft-versus-leukemia effects may contribute to the favorable outcome in RIST for ATL. Furthermore, it should be stressed that older patients who preferentially received RIST rather than CST achieved favorable survival comparable to younger patients (≥ 50 years: 61% vs < 50 years: 60%, P = .86). *Conclusion:* Allo-SCT for aggressive ATL remarkably improved the poor prognosis. Especially, RIST is a promising procedure for excellent outcome. We would suggest that RIST should be applied to all aggressive ATL patients eligible for allo-SCT, regardless of the age.

0977

LONGITUDINAL FOLLOW UP OF MINIMAL RESIDUAL DISEASE IN AML AFTER SCT WITH QUANTITATIVE EVALUATION OF WT1 GENE EXPRESSION

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Introduction. Wilms Tumor gene (WT1) overexpression is described in several oncological diseases including acute myeloid (AML)leukemias. The majority of AML patients don't have a suitable specific molecular marker for monitoring minimal residual disease(MRD). Quantification of WT1 in bone marrow samples can be useful as a marker of MRD after allogeneic stem cell transplantation (SCT) and can predict AML relapse.METHODS and RESULTS. We have evaluated, sequentially and using a quantitative RT-PCR technique (LeukemiaNET method), WT1 expression in 50 consecutive AML patients that overexpressed WT1 at diagnosis and that underwent allogeneic SCT at our centre. The cDNA level of WT-1 was detected in bone marrow samples at diagnosis at the time of transplant and after the allogeneic SCT. Samples of diagnosis showed high WT1 expression levels in all cases with a mean of 5570 (SD 4055) copies of WT1/10000 Abl, median 4600 (range 658-23913) copies WT1/10000 Abl. At transplant 34 pts (68%) were in complete cytologic remission (CcR) and 16 (32%) had refractory or relapsed AML. Bone marrow samples from pts in CcR at BMT showed significantly lower WT1 expression levels (mean 88±130), compared to the samples from pts with relapsed or refractory disease (mean 5727±4265) (P < 0,001). After BMT a rapid decline of WT1 expression levels was observed in all pts that achieved and/or maintained a condition of CcR, especially in those that were in CcR at SCT. After a median follow up of 11 mths from transplant, 10 out 50 pts relapsed (20 %) and all of them had high expression levels of WT1 before the cytological relapse. Three of these pts were successfully reinduced with DLI ± chemotherapy with a rapid reduction of WT1 levels.CONCLUSIONS. 1)In our experience there is a complete concordance between WT1 expression levels (measured by quantitative RT-PCR) and status of AML before and after SCT. 2)Our study confirms that longitudinal quantitative evaluation of WT1 after SCT may be useful as a non-specific leukemia marker (NSLM) for monitoring MRD and as a predictor of AML relapse. 3) Based on these results cases with an increase of WT1 levels after SCT and without GVHD should be candidate to DLI and/or discontinuation of immunosuppressive therapy.

0978

RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN HEMATOLOGICAL MALIGNANCIES: FACTORS IMPACTING ITS OCCURRENCE AND TREATMENT OPTIONS FOR A BETTER MANAGEMENT

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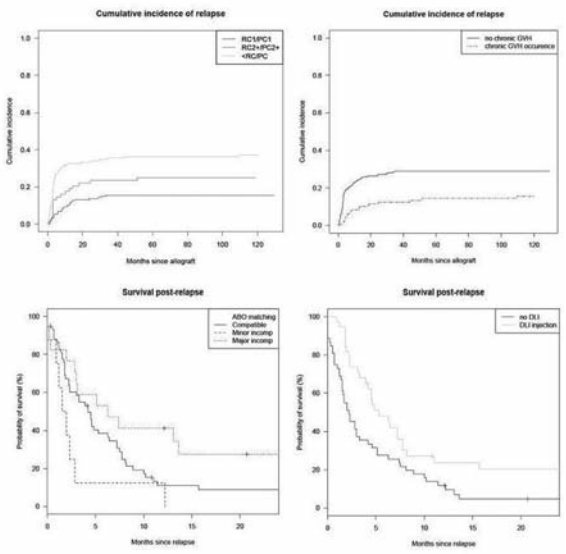
Introduction. Relapse remains a major cause of mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with hematological malignancies. **Aims.** To evaluate at a first time the different pre- and post-transplantation factors impacting the relapse occurrence after allo-HSCT, and at a second time, to evaluate factors impacting the survival post-relapse including the different treatment options. **Methods.** We have retrospectively studied the occurrence of relapse in 345 patients, 198 (57%) males and 147 (43%) females with a median age of 43 years (range17-66) who received allo-HSCT at our institution for hematological malignancies between years 2000 and 2011; 205 (59%) from siblings donors and 140 (41%) form unrelated donors. At transplantation, there were 148 (43%) patients in first complete response or first chronic phase (CR1/CP1), 66 (19%) in CR2/CP2 and 131 (38%) < CR2/CP2. Two hundred and six (60%) patients received a full intensity condi-

tioning and 139 (40%) a reduced intensity one. The different patients and transplantations characteristics will be communicated during the meeting. **Results.** After HSCT, 336 (97%) patients engrafted. The cumulative incidence of acute GVHD≥2at 3months was 35% (95%CI 32-37); the cumulative incidence of extensive and limited chronic GVHD at one year was the same 15% (95%CI 13-17). After a median follow-up of 11.4 months (range 4-129), the median overall survival (OS) for the whole population was 19 months (range 12-33) with a 2-years probability of 47% (95%CI 42-53). Eighty eight (25.5%) patients relapsed with a cumulative incidence at one and two years of 19% (95%CI 17-21) and 22% (95%CI 20-24) respectively. After relapse, 65 (74%) patients were treated [21 (32%) received donor lymphocyte infusion (DLI) alone, 21 (32%) chemotherapy alone, 14 (22%) DLI + chemotherapy and 9 (14%) received other treatment] and 23 (26%) were not treated due to deadly relapse. The median OS from relapse was 4 months (range 3-5) and the one year probability of OS in patients who relapsed was 21% (95%CI 14-31).The multivariate analysis studying the pre- and post-relapse variables on the survival after relapse showed a positive impact of ABO incompatibility [Major ABO incompatibility: HR=0.39 (0.16-0.9), p=0.03], a negative impact of disease status [HR=2.4 (1.3-4.4), p=0.003] and a positive survival outcome in patients receiving DLI [HR=0.5 (0.3-0.8), p=0.005].



Conclusions. Survival after relapse post-allo-HSCT is still very low reflecting the difficulty to find an optimal treatment, disease status at transplantation seem to have a long term effect while the use of DLI with or without chemotherapy can offer better results. In addition to the new chemotherapy molecules, immunotherapy should be used in order to enhance the graft-versus-leukemia effect not only after relapse, but also early in presence of a minimal residual disease.

0979

REDUCED INTENSITY CONDITIONING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH AGGRESSIVE CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Allo-HSCT remains the only curative option in patients with CLL. With the median age at presentation of 72 years, and the high TRM associated with myeloablative conditioning regimens, several groups have opted for a reduced-intensity conditioning (RIC) approach which appears to provide long-term disease control in a subset of patients with aggressive disease **Aims.** There have been recent reports suggesting that the use of pre-transplant alemtuzumab and/or T-cell depleted conditioning regimens may increase the risk of early relapse/progression. We therefore assessed the outcomes of CLL patients undergoing RIC allo-HSCT with a T-cell replete conditioning regimen in terms of TRM, acute and chronic GVHD, OS and relapse/progression post-transplant.**Methods**A retrospective analysis was performed on 19 consecutive patients with biologically aggressive CLL who underwent RIC allo-HSCT from NottinghamUniversityHospitalsand the RoyalMarsdenHospitalbetween 2008 and 2011. All patients received Fludarabine 90mg/m^2 and 2Gy TBI conditioning, with mycophenolate mofetil and cyclosporine for GVHD prophylaxis. Biologically aggressive disease was characterized by del(17p13) or del(11q23) cytogenetic abnormalities and/or purine analogue refractoriness and/or high-grade transformation.

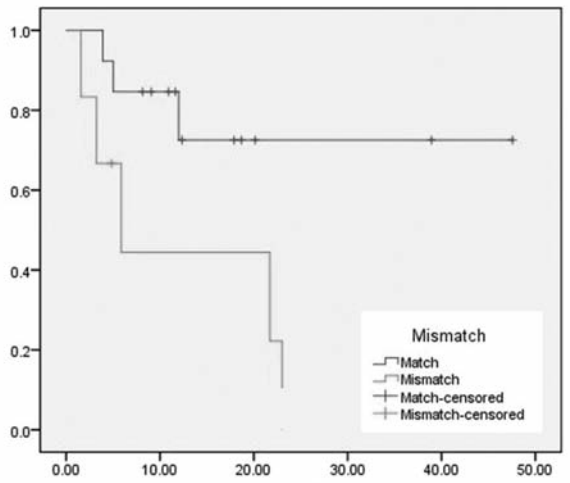

Figure 1. Overall survival comparing matched (sibling and unrelated donor) vs. mismatched donor allografts.

Results. Median age at the time of allo-HSCT was 59 years (range 48-69). Only 2 patients were female. Median duration from CLL diagnosis to allo-HSCT was 5.8 years (range 1-15). Patients received a median of 3 lines of chemotherapy pre-transplant (range 1-7). 13/19 patients had received purine analog-based chemotherapy of whom 12/13 were refractory, 15/19 had alkylating agents and 14/19 had alemtuzumab. Two patients had high-grade transformation during the course of their disease. On evaluation by FISH, 9 patients haddel(17p13), 5 haddel(11q23) and 2 haddel(13q14).At the time of allo-HSCT, 5 patients were in CR, 11 in PR and 3 had SD. 16/19 patients received unrelated donor allografts (10 matched by high-resolution typing at HLA-A, B, C, DRB1 and DQB1; 6 mismatched) and 3/19 received HLA-identical sibling grafts; 18/19 patients received PBSC.Patients were followed up for a median of 1 year (range 0.1-3.9). 11/19 patients remain alive, of whom 10/11 are in CR, and 1/11 in stable PR. OS at 1 year is 72% and 32% at 2 years, with a marked difference in survival between the HLA-matched (sibling and UD) vs. mismatched transplants (85% vs. 44% at 1 year, 72% vs. 16% at 2 years). This was due to a higher TRM in mismatched transplants. Acute GHVD occurred in 11/19 patients with 4/11 patients developing Grade 3-4 GVHD. 11/19 patients developed chronic GVHD, which was extensive in 6/11 patients. Viral reactivation occurred in 8/19 patients (5/8 CMV with one causing CMV disease, 2/8 HHV6 and 1/8 EBV). The use of alemtuzumab pre-HSCT, number of prior treatments, patient age or donor source were not predictive of outcome. **Conclusions.** In this cohort of patients with high risk CLL, degree of HLA matching was found to be the greatest predictor of OS following RIC allo-HSCT. Excellent results can be achieved with matched sibling and unrelated donors. Our results would suggest avoiding the use of mismatched donors in this setting.

0980

STEM CELL MOBILIZATION FOLLOWING G-CSF PLUS/MINUS PLERIXAFOR: A PREEMPTIVE MODEL OF PLERIXAFOR USE BASED ON DAY 4 CD34 LEVELS

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Plerixafor has been recently approved to enhance mobilization of peripheral blood stem cells (PBSC) in combination with G-CSF in patients with lymphoma and multiple myeloma. Prospective monitoring of CD34 levels on day 4 of G-CSF could allow identifying patients for whom the benefit derived from the enhanced mobilization capacity of plerixafor can be maximized. To uniformly manage these patients who appear to mobilize poorly, we present our preemptive algorithm of plerixafor use based on PB CD34 cell counts on day 4 of G-CSF as a target. Forty one patients (7 myeloma, 34 lymphoma; 24 men; median age 58 [27-69]; 68% failed prior mobilization attempts [1-4]) with suboptimal PB CD34 cell levels on day 4 of G-CSF steady-state mobilization and receiving plerixafor rescue treatment (CD34/µL <5, n=25; 5-10, n=13; 10-20, n=3) were compared with 130 coetaneous controls from the same institutions (53 myeloma, 77 lymphoma; 73 men; median age 56 [22-71]; 11% with prior failed mobilization attempts [1-2]) with day 4 CD34 level monitoring who did not receive plerixafor (CD34/µL <5, n=47; 5-10, n=20; 10-20, n=63). Plerixafor administration on day 4 significantly increased the PB CD34 level 4.7-fold±2.8, even for the subgroups with <10 CD34/µL (4.7-fold ±2.9) and <5 CD34/µL (5.3-fold ±3.2) compared to G-CSF alone (2±1.1 for all subgroups, p<0.001). In particular, patients with <10 CD34+ cells/µL on day 4, who are more likely to mobilize poorly, collected higher numbers of PBSC with plerixafor compared to G-CSF alone in the first apheresis (2.3 vs 0.6 CD34 x10^6/kg; p<0.001) and overall (2.9 vs 1.02 CD34x10^6/kg; p<0.001). Moreover, the preemptive use of plerixafor provided >=2x10^6/Kg CD34 cells in 74% of these patients compared to 27% with G-CSF alone (p<0.001). Even in patients with baseline PB CD34 levels <5/µL, plerixafor provided a CD34+ yield of 1.8±1.5 x10^6/kg in the first apheresis, and 2.4±1.5 x10^6/kg overall, successfully collecting >=2x10^6/kg CD34 cells in 68% of these patients vs 15% with G-CSF alone (p<0.001). In first mobilizations attempts, the addition of plerixafor in the evening before the first apheresis, significantly increased up to 92% (vs 28%, p<0.001) and 86% (vs 16%, p<0.001) the number of patients collecting overall >=2x10^6/kg CD34 cells when the PB CD34 on day 4 were <10/µL and <5/µL, respectively. There were no adverse drug-related events. Up to now, 29 plerixafor patients (71%) have received an autologous HSCT with a median CD34+ cell dose infused of 3.4 (1.6-7.2) x10^6/kg. Of note, all patients engrafted and reached >0.5x10^9/L neutrophils after a median of 12 (10-21) days and achieved a self-sustained platelet count >20 x10^9/L after a median of 15 (11-28) days. No cases of secondary graft failure were observed. Based on these data, it seems possible to establish an algorithm for preemptive plerixafor use based on day 4 PB CD34+ cell counts. This strategy may identify potential poor mobilizers (<10 CD34+ cells/µL on day 4) for whom the incremental benefit of plerixafor is maximized. This approach may translate into a reduction of remobilization attempts, preventing from treatment delays and improving cost-effectiveness and resources utilization.

0981

RECONSTITUTION OF THE T CELL COMPARTMENT AND THE THYMIC FUNCTION AFTER REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The thymus plays a pivotal role in generating naive T cells from hematopoietic precursors and long-term immune reconstitution after allografting. **Aims.** To asses the short- and long-term reconstitution of the CD4+ T cell compartment and the residual thymic activity in elderly patients undergoing allogeneic hematopoietic cell transplantation. **Methods.** The study population consisted of 63 consecutive recipients, median age 56 (r 23-67), of a T cell repleted allograft from siblings or unrelated donors after thiotepa/cyclophosphamide-based reduced-intensity or low-dose total body irradiation-based non-myeloablative conditionings. One patient underwent thymectomy 10 years before the allograft. The reconstitution of the CD4+ T cell compartment was evaluated by flow cytometry. Naïve, central memory, effector memory, and "revertant"CD4+ T cells were identified by co-expression of CD45RA and CD27, CD45RO and CD27, expression of CD45RO (CD27 negative), and co-expression of CD45RA and CDRO, respectively. Blood samples were drawn at baseline, and at 3, 6, 12, 18 and 24 months post-transplant. To quantify residual thymic function and to differ true naïve CD4+ from "revertant"CD4+ T cells, T-cell receptor excision circles (sjTRECs) were assessed by real time PCR at the same time points on DNA extracted from sorted CD4+ T cells. sjTRECs are episomal excision products of the T cell receptor gene rearrangements that occur in maturing thymocytes; do not replicate during mitosis and are diluted during cell division providing an excellent measure of thymic function during periods of immune reconstitution. Primary endpoint was the trend over time of sjTRECs copy number/ 100 ng DNA; secondary endpoints were trends over time of naïve, central memory, effector memory, and revertant CD4+ T cell numbers after transplant, and their potential modifications by patient- and transplant-related independent variables. Univariate and multivariate analyses included: age at transplant (>56 vs. ≤56 years), gender (female vs. male), conditioning regimen (reduced-intensity vs. non-myeloablative), donor type (unrelated donor vs. sibling donor), disease status at transplant (less than CR vs. CR), occurrence of acute graft-vs.-host disease (GVHD) and chronic GVHD, number of CD3+ and CD34+ cells/ kg recipient body weight infused.*Results.* Naïve, central memory, effector memory, and, to a far lesser extent, "revertant"CD4+ T cell values showed a gradual significant increase throughout the study period. However these values were lower than those observed in healthy donors. A gradual statistically significant increase in sjTRECs from baseline up to 2 years post-transplant was also observed (p<0,001). Importantly, siTRECs levels were undetectable in the patient who had undergo thymectomy prior to transplant. By multivariate analysis, a significantly lower sjTRECs increase was seen in patients conditioned with a reduced-intensity rather than a non-myeloablative regimen (p=0,008), transplanted from an unrelated donor (p=0,002), and in those who developed chronic GVHD (p=0,029). By contrast, no differences were seen in patients who developed acute GVHD from those who did not. **Conclusions.**Effective residual thymic activity contributing to long-term immune reconstitution was seen in this elderly patient cohort. The thymus remains sensitive to chemotherapy-induced damage and is the target of chronic GVHD but not acute GVHD.

0982

ETANERCEPT FOR STEROID REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE: THE SCOTTISH EXPERIENCE

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Background. Etanercept is a recombinant human tumour necrosis factor alpha (TNF-α) receptor fusion protein that inhibits the action of TNF-α, a cytokine crucial in the pathogenesis of acute GvHD. Etanercept has shown potential benefit in treating steroid refractory acute GvHD. **Aims.** We report the experience of using Etanercept for the treatment of acute GvHD in the Scottish alternative donor HSCT unit. **Methods.** This study is a retrospective analysis of patients who received Etanercept for acute GvHD between May 2008- August 2011. Transplant demographics, prior treatment for GvHD, Etanercept treatment, complications and outcomes were assessed. Etanercept was administered as 25mg subcutaneously twice weekly for 4 weeks, followed by 25mg weekly for 4 weeks. **Results.** 19 patients with acute GvHD were identified; 7 classical acute GvHD (2 post DLI), 9 late onset acute GvHD, 3 acute pulmonary GvHD/Idiopathic pneumonia syndrome. Patients with classical acute GvHD all had grade III/IV disease and were refractory to corticosteroids, while late onset patients had grade II disease and had had partial response to previous therapies. Diseases were AML (n=6), ALL (n=3), CLL/Lymphoma (n=6),CML (n=2),MF (n=1), Fanconi Anaemia (n=1). Stem cell source was PBSC in all cases. Conditioning was myeloablative in ten cases and reduced intensity (RIC) in nine. GvHD prophylaxis was with Ciclosporin and short course methotrexate in the myeloablative patients and ciclosporin alone in the RIC patients. Donor was a sibling in eight patients, a volunteer unrelated donor in nine (10/10 (1 second transplant) (n=6), 9/10 (n=2), 8/10 (n=1)), and double umbilical cord blood in two patients (4/6 x 4). The average cell dose was 6.1x10^6/kg. All had evidence of GvHD at multiple sites; gastrointestinal disease was most common n=15, liver n=12, skin n=12, lung n=3. The median number of prior therapies

was four (1-6). The median days from transplant to Etanercept treatment was 139 (47-4316) and from onset of GvHD was 68 (10-3986). The median number of doses of etanercept was eight (4-12) and concominant therapies was three (2-5). Concomitant therapies included corticosteroids ,ciclosporin, tacrolimus, MMF,photopheresis and BudesonideResponses were seen in 6/9 (66%) late onset patients (1CR, 5PR); 3/7 (43%) classical acute (3PR), and 0/3 acute lung damage. All patients who responded were able to reduce or stop steroids and had improvement of the gastrointestinal component of their disease. There were 4 long term responders in the late acute GvHD group (3 alive, 1 died MI). Unfortunately, in the classical acute GvHD patients, all responders died of infection, with recurrence of GvHD in 2/3. **Conclusions.** n our hands, Etanercept was ineffective in treating acute pulmonary injury < 100 days post transplant and there was limited durable efficacy in those patients with severe grade III/IV classical acute GvHD with true steroid refractoriness. However, valuable, durable responses were seen in patients with late onset acute GvHD, especially affecting GI tract, where steroid doses were able to be reduced or stopped.

0983

AUTOLOGOUS STEM CELL TRANSPLANTATION AS SALVAGE TREATMENT FOR PRIMARY REFRACTORY/RELAPSED HODGKIN AND NON HODGKIN LYMPHOMA: LONG-TERM OUTCOME IN OVER 300 PATIENTS

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Aims. To analyze clinical outcome and significant prognostic factors for overall survival (OS) and progression free survival (PFS) in patients(pts) with Hodgkin (HD) and non Hodgkin lymphoma (NHL) undergoing autologous stem-cell transplantation (ASCT). **Methods.** Between October, 1987 and November, 2010 330 pts with HL and NHL that were not controlled by conventional chemotherapy underwent high-dose chemotherapy and ASCT by our transplant centre.Results. 216 pts were NHL and 114 HD, 183 were male, median age was 39 years (range, 15 to 67 years). At the time of ASCT 161 (125 NHL, 36 HDG) were in complete remission (CR), 72 (55 NHL, 17 HDG) were in partial remission (PR) and 97 (36 NHL, 61 HDG) presented a resistant disease (RD). All pts received chemotherapy with high-dose regimens: 126 were treated with BEAM, 119 with ICE, 67 with Mitoxantrone-Melphalan and 18 were treated with other type of therapy. To proceed to ASCT, pts had to have adequate cardiac, pulmonary, hepatic, and renal function. The median time to engrafment was 11 (range 7-20) days for absolute neutrophil count (>500), 11 (range 5-40) days for platelets (20.000). After ASCT 204 pts were in CR, 44 were in PR and 82 presented a RD. With a median period of observation of 61 months (CI 95% 54-67) 221 pts are alive and 109 are death, the overall survival (OS) calculated with the Kaplan Mayer method is 59%. With a median period of observation of 49 months (CI 95% 40-52) 162 pts are free from progression and the progression free survival (PFS) is 49%. No differences in OS or PFS were observed between NHL and HD (respectively 67% vs 55% and 51% vs 46%). In NHL OS was significantly superior in pts arrived at transplant in CR (p:.003), and in pts treated with no more than two previous chemotherapy (p:.002); in HD, OS was affected only by the CR status at transplant (p:.01). Similarly, in NHL PFS was significantly affected by CR status at transplant (p:.0000) and to have received less than three previous therapy (p:.001); in HD only CR status at transplant affected significantly the PFS (p:.0004). **Conclusions.** this monocentric data confirm the importance of ASCT as salvage treatment either in NHL or in HD, in NHL it seems better to use this strategy earlier in the history of the disease while in HD even ptstreated with several therapy, reaching a response before transplant, obtain excellent results. Finally chemo-sensibility remains the most important factor for prognosis.

0984

HEMATOLOGICAL RECOVERY AFTER INTRA-BONE CORD-BLOOD TRANSPLANTATION IS INFLUENCED BY NUMBER OF TOTAL NUCLEATED CELLS AND CD34 CELLS, BUT NOT INFLUENCED BY ORIGIN OF BANKS OR TIME OF CRYOPRESERVATION

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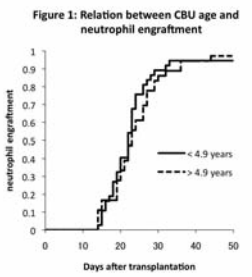
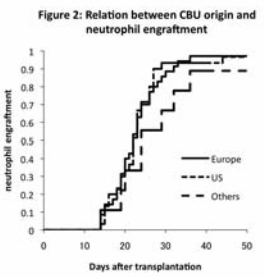
Background. Cord-blood transplantation (CBT) is an effective option for both malignant and non-malignant hematological diseases and its increasing number in the last years has enlarged opportunities to use cord-blood units (CBUs) trough international borders in order to maximize chances of identifying optimal CBUs. Since not all banks are accreditated by a global standard regarding CB quality control, the quality of CBUs can be affected by the origin of banks. In addition, little information is available on cord blood engraftment capacity after long-term cryopreservation. In this study, we evaluated whether the origin and the age of CBUs may influence the quality of CBUs and post-transplant hematological recovery. **Methods.** CBTs with single unit were performed at San Martino Hospital in Genoa for hematological malignancies using an intra-bone CBT technique. Just before infusion, a small sample of the CBU was obtained and post-thawing number of total nucleated cells (TNCs), CD34-positive cells, and colony-forming cells (CFCs) were characterized. Median pre-freezing number of TNCs and CD34-positive cells were 2.32 (1.15 - 4.52) x 10^9 and 9.3 (2.4 - 27.24) x 10^6, respectively. Recovery of neutrophil and platelet were compared according to the origin and the age of each CBU.Results. 95 CBUs were tested. The origin of CBUs was Europe (n = 45), the United States (n = 40), and other regions (n = 10). Median age of CBUs was 4.9 (0.4 - 11.8) years; CBUs originated from European banks were significantly older than CBUs from other regions (5.9 years vs 3.8 years, p value of unpaired t-test < 0.001). Median post-thawing number of TNCs, CD34-positive cells, and CFCs did not differ considering older (> 4.9 years) or younger CBUs (< 4.9 years) or the origin of the banks. Median time of neutrophil and platelet engraftment was as follows: younger CBUs: 24 and 34 days, older CBUs: 25 and 35 days, respectively (Fig.1). Hematological recovery was not influenced by the bank origin of CBUs as well, namely, rate and time of neutrophil and platelet engraftment was, 22 and 34.5 days (Europe), 22 and 35 days (the United States), 24 and 42 days (the other region), respectively (Fig.2). In contrast, days of neutrophil and platelet recovery were strongly correlated with the number of TNCs and CD34-positive cells per recipient's body weight.Conclusions: Although time of cryopreservation depends on origin of banks, the origin and the age of CBUs have little impact on post-thawing values of TNCs, CD34-positive cells, and CFCs. In addition, hematological recovery after CBT was influenced by number of TNCs and CD34-positive cells per recipient's body weight, but not influenced by the origin or the age of CBUs. These data suggest that quality control of CBU is working well regardless of the bank origin.





0985

COMPARISON OF VARIOUS HEMATOPOIETIC STEM CELL MOBILIZATION REGIMENS IN PATIENTS WITH LYMPHOMA AND MYELOMA

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Background. A little-known mobilization regimen, IEV (Ifosfamide, epirubicin, and etoposide), has been used for hematopoietic stem cell mobilization recently as well as standard mobilization protocols. But retrospective and prospective studies that compare the efficacy and toxicity of hematopoietic stem cell mobilization regimens are needed to determine the least toxic and most effective regimen. **Aims.** The aim of this study was to compare the stem cell mobilization success, the factors affecting the stem cell sufficiency, and the toxicities of well-known and effective cyclophosphamide or platin-based regimens and IEV regimen in MM, HL, NHL patients who were planned to undergo autologous hematopoietic stem cell transplantation. **Methods.** A total of 203 (119 male and 84 females) patients who underwent peripheral stem cell mobilization before autologous hematopoietic stem cell transplantation (SCT) between January 2000 and April 2010, with the diagnosis of multiple myeloma (MM, n= 94), Hodgkin lymphoma (HL, n= 37) and non-Hodgkin lymphoma (NHL, n= 72) were retrospectively analyzed. G-CSF (filgrastim, lenograstim) 10 mcg/kg/day was administered to all patients in addition to mobilization protocol. Successful mobilization was defined as collection of ≥3x10^6 CD34 (+) cells/kg after apheresis for single autologous transplantation and ≥5x10^6 CD34 (+) cells/kg for double autologous transplantation. **Results.** There were no significant differences between platinum- or cyclophosphamide-based mobilization regimens and IEV

chemotherapy schema in terms of success of peripheral stem cell harvest. Peripheral blood stem cell yield was significantly higher in ESHAP group than that of DHAP and ICE groups (median 12.7 vs 6.6 vs 6.9 x10^6 CD34 (+) cells/kg, respectively, p=0.01). There were no significant differences between cyclophosphamide-based regimens and IEV regimen in patients tandem transplantation planned. Low and high dose cyclophosphamide provided comparable amount of stem cells collected. Grade 3-4 toxicity profiles were similar between mobilization groups. Regression analysis did not reveal any significant independent predictor for stem cell mobilization success or yield of collected stem cells. **Conclusions.** Although there was not any significant differences between the mobilization procedures, use of ESHAP may be preferable for peripheral stem cell mobilization in lymphoma patients whom unsuccessful mobilization expected or back-up stem cell collection needed. Because of comparable efficiency and toxicity, IEV may be an alternative regimen for stem cell mobilization.

0986

ALLOGENEIC STEM CELL TRANSPLANTATION FOR RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA: A SINGLE CENTER EXPERIENCE

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Background. Patients with acute myeloid leukemia (AML) who are refractory to induction therapy or relapse after remission have a 1 year survival probability lower than 30%. Because of this dismal prognosis, in these patients allogeneic HSCT is considered the recommended therapy.AimsWe retrospectively analyzed 78 patients with relapsed (n=38), primary refractory (n=34) or untreated (n=6) AML who underwent allogeneic HSCT at our Institution between 2002 and 2011, to verify outcome and to identify factors that can affect long term outcome. MethodsMedian age at HSCT was 48 (range: 18-72) years, with 21 patients (27%) who received transplant when aged over than 60 years. For the 38 relapsed patients, median duration of CR was 6.5 months. Allogeneic HSCT was performed at a median of 8 (range: 1-179) months from diagnosis; in relapsed patients, median time from relapse to transplant was 3 months. Forty-two patients (54%) received grafts from a sibling donor and 36 (46%) from a matched unrelated donors (MUD). In 53 patients (68%) stem cells source was peripheral blood (PB), while bone marrow (BM) stem cells were used in 25 cases. Myeloablative conditioning regimens were used in 48 patients (24 siblings, 24 MUD): BuCy in 43 cases, TBI-based regimens in 5. Thirty patients (18 siblings, 12 MUD) received reduced-intensity conditioning.ResultsAcute GVHD developed in 37/78 patients (47%), with a similar incidence in sibling (43%) or MUD (53%) transplants and in recipients of BM (56%) or PB (43%) stem cells. Chronic GVHD occurred in 19 of the 65 evaluable patients (29%), with an almost identical incidence in MUD (30%) and sibling recipients (29%) and according to stem cell source (BM=30%, PB=29%). With a median follow-up time of 5 years, 13 of the 78 transplanted patients (17%) are alive and in CR, while 64 patients have died. The mortality rate was similar in the MUD (31/36, 86%) and sibling (33/42, 79%) transplant. Main causes of death were disease recurrence (37 patients, 58%), infections (10 patients, 16%) and GVHD (6 patients, 9%). Cumulative 1-year non-relapse mortality (NRM) was 27%, with infections (10 cases, 37%) and GVHD (6 cases, 22%) being the two most common causes of NRM. Analyzing clinical factors before transplantation, a Karnovsky performance status (PS) ≥ 80% and a full matched donor were associated with a better outcome of HSCT. Considering the post-transplant variables, full donor myeloid chimerism at recovery and development of chronic GVHD were associated with a longer overall survival. Grouping patients according to the two pre-transplant factors (PS and HLA compatibility), we identified three cohorts with significant differences in death probability: 5-years survival probability was 75%, 24% and 0% in patients with no, one or two risk factors (P = 0.0001).Summary / conclusionsOur data confirm the capacity of allogeneic transplant to prolong survival in a significant proportion of extremely high-risk AML patients. Further studies are warranted to establish the best conditioning regimen and identify strategies improving graft versus leukemia effect.

0987

PREDICTORS OF PROLONGED SURVIVAL AND LONG-TERM OUTCOME AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION WITH RIC IN PATIENTS WITH MULTIPLE MYELOMA.

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Allogeneic stem cell transplantation (allo-SCT) has been used in the hope of harnessing the curative potential of the graft-versus-myeloma effect. This study examines the long-term outcomes of a cohort of patients with myeloma who were treated with reduced-intensity conditioning (RIC) regimens after a minimum follow-up of 5 years at the INSTITUT PAOLI CALMETTES Cancer Centre.A total of 53 patients with multiple myeloma (MM) who received allo-SCT between January 2000 and January 2007 were identified. Use of ATG, patient age, number of prior auto-SCT, delay between auto and allo-SCT, disease status at allo-SCT, occurrence of grade II-IV acute or chronic GVHD were included as predictive factors for OS, PFS and TRM. Variables with p<0.10 were included in multivariate analysis. The median follow-up of alive patients was 82 months (48-137). Median age of MM patients was 50 (28-70) years. 51 patients (96%) received transplant from a sibling donor. The median time between diagnosis and allo-SCT was 34 months (6-161), 51 patients (96%) received at least one auto-SCT; 24 patients (45%) received a tandem auto-allo-SCT. and the median time between auto-SCT and allo-SCT was 10 months (1-89). Conditioning regimens were Melphalan-based for all Auto-SCT patients, whereas the allo-SCT patients received Fludarabine, busulfan and Anti thymoglobulin (ATG)-based (58%), or Fludarabine and Total body irradiation (TBI) based (38%) or other (4%).At last follow-up, there are 21 patients (40%) who are alive >5 years post RIC allo-SCT; of those 14 (26%) are in first complete remission (CR), and 4 patients (8%) in second CR after donor lymphocytes injection or re-induction with the new drugs (bortezomib or lenalidomide) after allo-SCT. Only 4 patients (19%) of these long survivors received one of the new antimyeloma drugs as induction or relapse treatment before allo-SCT.Cumulative incidence of grade II-IV acute graft-versus-host disease (aGVHD) was 36% and the cumulative incidence of chronic GVHD (cGVHD) was 55% at 2 years. Acute GVHD had not influence on overall survival (OS) or progression-free survival (PFS), while cGVHD had significant protective effect on PFS: HR=0.42 (0.22-0.78, p=0.007). OS was 52% (39-65) at 5 years and 32% (16-48) at 10 years. The PFS was 26% (14-38) at 5 years and 24% (12-36) at 10 years. Treatment-related mortality (TRM) at 1 year was 20% (9-31).Only disease status and occurrence of cGVHD were significantly associated with PFS; acute GVHD was correlated with higher TRM. No variables were associated with OS.In conclusion, we observe that long-term disease control can be expected in a subset of MM patients undergoing RIC allo-SCT. With OS and PFS after 10 years of 32% and 24 % respectively, and the survival curves after RIC allo-SCT stabilizes with time.

0988

RABBIT-DERIVED ATG BUT NOT HORSE-DERIVED ATG IN THE CONDITIONING INDUCES A POST TRANSPLANT IN VIVO IMBALANCE BETWEEN B AND T-CELL RECOVERY RESULTING IN HIGH RISK OF EBV ASSOCIATED PTLD

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After reduced intensity conditioning with fludarabin, busulphan and Lymphoglobulin (horse-derived ATG, hATG) followed by alemtuzumab-based T-cell depleted (TCD) allogeneic stem cell transplantation (alloSCT), CMV reactivations were frequently observed in our patient cohort, but no EBV-associated post-transplant lymphoproliferative disease (PTLD). After the enforced replacement of hATG by Thymoglobulin (rabbit-derived ATG, rATG) CMV complications remained similar but an unacceptable incidence of 26% of early EBV-PTLD was observed.In this study, we analyzed the cause of this immune escape of EBV-infected B-cells by measuring antibody levels and specificity in relation to B and T-cell recovery early after transplantation.During conditioning 16 patients received rATG (total 8 or 14 mg/kg) for in vivo TCD. This cohort was compared to 16 patients conditioned with hATG for in vivo TCD (total 20 or 40 mg/kg).Total serum ATG levels after alloSCT as measured with species-specific ELISA at 3 and 6 weeks after alloSCT were similar for the hATG (43 and 15 ug/mL) and the rATG cohort (41 and 21 ug/mL). Next, specific ex-vivo reactivity of the circulating ATG with human B and T-cells was measured by a flow-

cytometry-based method. In serum from hATG treated patients, a high and sustained specific anti-B-cell reactivity was detected, which was dominant over anti-T-cell reactivity, resulting in expansion of memory T-cells which include EBV-specific T-cells prior to B-cell recovery. In contrast, serum of rATG treated patients contained only marginal levels of antibodies showing anti-B-cell reactivity, but dominant anti-T-cell reactivity resulting in early B-cell expansion in the absence of T-cell recovery. Interestingly, when tested directly out of the vial both ATG products showed similar dominant functional anti-T-cell reactivity over anti-B-cell reactivity as demonstrated using cell lineage-specific flow-cytometry and in vitro CDC assays. Interestingly, when in-vivo absorption of lineage-specific reactivity by circulating cells was mimicked in-vitro by co-incubation of a fixed amount of ATG to increasing amounts of peripheral blood mononuclear cells (PBMC) from healthy donors a similar difference was observed between rATG and hATG. Whereas the anti-B-cell reactivity was easily absorbed from the rATG already by low numbers of PBMC (<50x10^6), sustained anti-B-cell reactivity was present in hATG even after absorption with up to 300x10^6 PBMC. Absorption experiments with purified T and B-cells suggest a higher component of B-cell-reactive antibodies with no cross-reactivity for T-cells in hATG compared to rATG, explaining their longer persistence.In conclusion, after treatment with rATG, high levels of specific anti-T-cell reactivity was found up to 9 weeks after alloSCT, whereas anti-B-cell reactivity was only detectable early after transplant, allowing B-cell outgrowth and EBV-associated PTLD in the absence of EBV-specific T-cell control. In contrast, in vivo TCD with hATG resulted in dominant persistence of anti-B-cell reactivity up to 9 weeks after transplant. In-vitro absorption experiments suggest that the differential persistence of anti-B-cell reactivity after conditioning with hATG versus rATG is caused by differences in cell lineage specificity between the polyclonal ATG products.

0989

MIPI SCORE AND DAY-15 POST TRANSPLANT LYMPHOCYTE COUNT PREDICT SURVIVAL IN PATIENTS WITH MANTLE CELL LYMPHOMA TREATED WITH RITUXIMAB AND HYPERCVAD FOLLOWED BY BUSULPHAN AND MELPHALAN ASCT

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Background. Improved complete remission (CR) and overall survival (OS) rates have been reported in MCL (Mantle cell lymphoma) patients treated with Rituximab-HyperCVAD regimen (Romaguera JE et al. J clin Oncol 2005; 23: 7013-7023). ASCT (autologous stem cell transplantation) provides a further incremental improvement (Ritchie DS et al. Ann Hematol 2006; 86:101-105). MIPI (MCL international prognostic index) predicts outcome in non-ASCT treatment of MCL, but was reported to be not prognostic in patients treated with R-HyperCVAD (Shah, J J et al. Blood 2008; 112:2583-2583). ALC (absolute lymphocyte count) recovery after ASCT predict OS and PFS (progression free survival) in MCL patients treated predominantly with CHOP/CHOP-like regimens (Joao C, et al. BMT 2006; 37:865-871), but has not been assessed in those treated with R-HyperCVAD and ASCT.**Aims.** To analyse the impact of MIPI and ALC on day +15 after ASCT (ALC15) on patient outcome after treatment with Rituximab-HyperCVAD followed by ASCT with Busulphan(Bu) and Melphalan(Mel) conditioning as the initial therapy in patients with MCL. **Methods.** We analysed on an intention to treat basis, the outcome of 28 patients with MCL under the age of 65 enrolled in our treatment protocol with R-HyperCVAD followed by BuMel ASCT. One patient in CR post R-HyperCVAD refused ASCT. MIPI was calculated from pre-treatment variables. ALC15 was obtained from serial blood counts after ASCT. Informed written consent was taken from all patients.

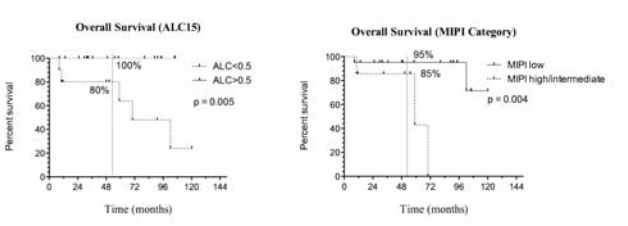

Figure 1. Overall Survival by ALC 15 and MIPI Category.

Results. CR was achieved after R-HyperCVAD in 92.6% of the patients. 100% of pts were in CR after ASCT. At a median follow up of 53months, estimated EFS (Event Free Survival) and OS were 68 % and 92.7% respectively. Because of low numbers patients with intermediate and high MIPI were analysed as a single group. Median ALC15 for the whole group was 0. 5x10^9/L. There were no significant differences between the two groups (ALC15>0.5 and ALC15 <0.5). EFS and OS rates at 53months in patients with ALC15 >0.5x10^9/L (n=17) were 94% and 100% compared to 34% and 80% in those with ALC15 <0.5x10^9/L (p=0.02 and 0.005 respectively). These rates in the patients with low MIPI (n=21) were 81% and 95% compared to 34% and 85% in those with intermediate/high MIPI (p=0.03 and 0.004 respectively). Transplant related mortality occurred in one patient by day 100. There have been no therapy-related MDS or AML in these patients. **Conclusions**. Treatment with Rituximab+Hyper-CVAD followed by ASCT with Busulphan-Melphalan conditioning achieves high CR rates and durable remissions in patients with MCL, in particular those with low MIPI and early ALC recovery. Patients with high MIPI or poor ALC recovery justify consideration of post-ASCT maintenance therapty.

0990

LONG-TERM FOLLOW-UP ON THE EFFECT OF THIOTEPA DOSE-INTENSITY IN THE CONDITIONING FOR ALLOGENEIC TRANSPLANTATION IN HEMATOLOGIC MALIGNANCIES

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Background. Thiotepa-fludarabine-cyclophosphamide (Thio-Flu-Cy) is an effective and reliable conditioning regimen for allogeneic transplantation (alloSCT). The intensity of Thio-Flu-Cy can be modulated varying Thiotepa dose based on patient age and comorbidities. **Aims.** This study was aimed at assessing whether varying the dose-intensity of Thiotepa in the conditioning has an effect on engraftment, survival, and graft-versus-host disease (GVHD). **Methods.** Eighty-seven patients with hematologic malignancies received peripheral blood stem cells (PBSC) at our centre between 2001-2011 from HLA identical siblings (77%), mismatched siblings (14%), or matched unrelated donors (9%) after Thio-Flu-Cy conditioning. Thio-Flu-Cy consisted of Thiotepa 5-15mg/kg (total dose) at days -6 and -5, Fludarabine 30mg/m^2/day and Cyclophosphamide 30mg/kg/day at days -4 and -3. The dose of Thiotepa was 5, 10, 12, and 15mg/kg for 13%, 39%, 15%, and 33% of patients, respectively. MUD or mismatched sibling recipients received rabbit anti-thymocyte globulin (rATG) 5-7.5mg/kg (total dose) at days -3 and -2, or alemtuzumab 7.5-15mg/m^2 at day -2. All patients received short-course methotrexate and cyclosporine for GVHD prophylaxis. Patients had non-Hodgkin (51%) or Hodgkin lymphoma (26%), multiple myeloma (16%), or AML (7%). At alloSCT, 47% of patients had >50 years, 51% were in CR, 34% in PR, 5% in SD, and 10% in PD. Thiotepa >10mg/kg was most frequently given to patients aged <=50 years and to those with residual disease at alloSCT (test for trend, p<0.001 and p=0.002). **Results**. Myeloablation occurred at day +2 (range, -3 to +7). Neutrophil and platelet engraftment (>500/mcl and >20000/mcl) occurred at day +12 (range, 5-21) and +13 (range, 9-33), and were not significantly affected by Thiotepa dose. All patients had full-donor chimerism by day +60. Median follow-up was 6.4 years (range, 0.2-10.4). Non-relapse mortality (NRM) was 7% at 100 days, 11% at 1 and 13% at 3 years. Relapse incidence was 44% at 3 and 47% at 5 years. Thiotepa dose did not affect NRM and relapse. Relapse was increased by disease burden at alloSCT (p=0.009). PFS was 43% at 3 and 39% at 5 years, OS was 63% at 3 and 60% at 5 years of follow-up. Multivariate analysis of PFS and OS included Thiotepa dose, disease status at alloSCT, rATG or alemtuzumab, and donor type (HLA identical sibling, mismatched sibling, or MUD). Disease status at alloSCT impacted PFS and OS (p<0.001 and p=0.01). Thiotepa dose and the other factors analyzed did not affect PFS and OS. The cumulative incidence of aGVHD was 35%. Thiotepa >10mg/kg increased the incidence of aGVHD compared to lower doses (48% vs 22%, p=0.01). The cumulative incidence of cGVHD was 28% at 1 and 40% at 3 years (extensive cGVHD, 11% and 14%). cGVHD was not affected by Thiotepa dose. Donor type did not impact aGVHD (p=0.39) and cGVHD (p=0.24). **Conclusions.** Thiotepa doses >10mg/kg in Thio-Flu-Cy conditioning are associated with an increased risk of aGVHD without improving relapse incidence, PFS and OS. Survival and relapse are independently affected only by disease status at alloSCT. Thus, Thiotepa at doses <=10mg/kg should be preferred even in case of residual disease when using Thio-Flu-Cy.

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RISK-BASED STRATIFIED GVHD PROPHYLAXIS IN ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION OF ACUTE MYELOID LEUKEMIA PATIENTS

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The general perception is that an unrelated donor HSCT, especially an HLA-mismatched unrelated donor HSCT presents greater risks in terms of graft-versus-host disease (GVHD) and non-relapse mortality (NRM) than matched sibling donor (MSD) HSCT. However, differences in post-transplant outcomes between MSD and unrelated donor PBSCT should be analyzed independently from the BMT setting in single-disease entity patients. Our center has adapted risk-based stratified GVHD prophylaxis according to the donor type and HLA matching degree in allo-PBSCT setting. In MSD transplants, patients received a short course of methotrexate in combination with cyclosporine, while tacrolimus and methotrexated were used in HLA-matched unrelated donor (MUD) transplants. In HLA-mismatched unrelated donor (MMUD) transplants, 1.25 mg/kg/day of anti-thymocyte globulin (ATG; Thymoglobulin, Genzyme Transplant) was infused for 2 days (D-2 and D-1) in addition to tacrolimus and methotrexate for GVHD prophylaxis. One hundred four consecutive AML patients who underwent allo-PBSCT were included in this analytic study. Fifty four patients (51%) received PBSCT from MSD, while 29 patients (28%) received PBSCT from MUD and 21 patients (20 %) received PBSCT from MMUD. The median follow-up duration after transplant was 19.4 months (range, 0.3-71.3). The median follow-up for living patients was 34.7 months (range, 6.5-71.3). Cumulative incidence of grade 2-4 acute graft-versus-host-disease (aGVHD) at 100 days was 22.6% in MSD transplant, 44.8% in MUD transplant, and 25.3% in MMUD transplant. MUD transplant showed the higher risk of grade 2-4 aGVHD compared to MSD transplant (P=0.028), while there was no difference between MMUD that used ATG and MSD transplant in terms of grade 2-4 aGVHD risk (P=0.932). Cumulative incidence of chronic GVHD (cGVHD) at 3 years was 70.5% in MSD, 63.4% in MUD, and 42.1% in MMUD. There was a trend towards lower risk of cGVHD in MMUD transplant compared to MSD transplant (P=0.092). Relapse incidence at 3 years was 16.7% in MSD transplant, 23.4% in MUD transplant, and 19.1% in MMUD transplant (P=0.562). Relapse risk did not differ between the each group. NRM at 3 years was 29.9% in MSD transplant, 21.1% in MUD transplant, and 30.2% in MMUD transplant (P=0.666). Disease free survival (DFS) and overall survival (OS) were 53.4% and 61.1% in MSD transplant, 61.7% and 65.1% in MUD transplant, 50.8% and 52.8% in MMUD transplant, respectively. DFS and OS also did not differ significantly between the each group. The analyses showed that ATG was able to overcome the high risk of acute and chronic GVHD in MMUD transplant and make comparable transplant outcomes between MSD and MMUD transplants. This present study demonstrates outcomes of MSD transplant and unrelated donor transplant for AML can be comparable in a allogeneic PBSCT setting if an adequate GVHD prophylaxis strategy is applied in unrelated donor PBSCT and the degree of HLA mismatch is not significant at the level of high resolution HLA typing.

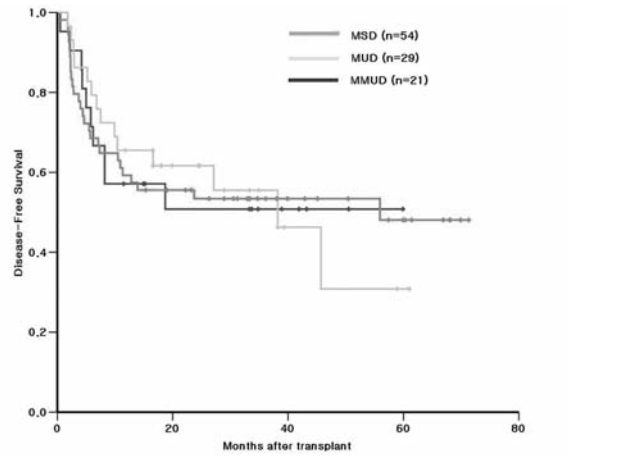

Figure 1. Disease-Free Survival in AML patients who adapted risk-based stratified GVHD prophylaxis in allogeneic peripheral stem cell transplantation.

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COMPLETE RESPONSE AFTER HIGH-DOSE THERAPY WITH AUTOLOGOUS STEM CELL SUPPORT IS THE ONLY IMPORTANT PREDICTIVE FACTOR FOR SURVIVAL

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Background. High-dose chemotherapy with autologous stem cell support (HD/ASCT) is the standard treatment for young patients with multiple myeloma (MM). **Aims.** In this study, we evaluated the relationship between the depth of HD/ASCT response and survival rates and examined whether the primary therapy with bortezomib-containing regimen was a factor for survival. **Methods.** We analyzed the clinical data of 129 patients treated with HD/ASCT in three institutions from 2000 to 2011. Every patient received two cycles of high dose dexamethasone. Then we treated the patients who showed response ≥PR with VAD (vincristine, adriamycin, dexamethasone) and treated those who had responses. **Results.** Clinical data of 129 patients were evaluated for their treatment responses based on previous their medical records. Their median age was 54 years old (range: 35-65 years) and 69 patients (53.5%) were male. Among these patients, 71 % of them (92 of 129 pts) were treated with bortezomib-containing regimen and 29% of them (37 of 129 pts) were treated with VAD before HD/ASCT. The response rates after PT were 27% CR, 17% VGPR, 43% PR. And post-HD/ASCT response rates were 64% CR, 17% VGPR, 10% PR. Median follow-up durations were 36.28 months (range, 5.52~145.33 months). Five-year overall survival (OS) rates and 5-year progression-free survival (PFS) rates were 58% and 25%. ß2-microglobulin and post-HD/ASCT CR are the strong predictive factors for OS (5-year OS rate; 79.3% and 41.2% in ß2-microglobulin <3.5 and≥3.5, respectively; p=0.01), (5-year OS rate; 73.5% and 31.6% in CR andWe evaluated the response rates and survival rates between bortezomib-containing regimen and VAD treated-group. The response rates after VAD were 27% CR, 16% VGPR, 49% PR and the response rates after bortezomib-containing treatment were 27% CR, 17% VGPR, 40% PR. In two groups, the response rates were similar (p=0.86). After HD/ASCT, the rates of OS were not significantly different in two groups (5-year OS rate; 48% in VAD group vs 65% in bortezomib-containing group respectively; p=0.26) The percentage of ≥ grade III peripheral neuropathy was 25% in bortezomib-containing regimen group but was not observed in VAD group (p= 0.01). And the number of collected CD34+ cells was not inferior in VAD group when compared to bortezomib-containing group (median CD34+cell counts/one harvest; 5.4 (10^6/kg) in VAD group vs 6.1(10^6/kg) in bortezomib-containing regimen respectively; p=0.52). **Conclusions**. We concluded that CR after HD/ASCT is the primary factor which impacts the survival rates of the patients. And when the patients show good responses to dexamethasone, VAD is still a feasible primary therapy in MM before HD/ASCT.

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EFFECT OF MESENCHYMAL STEM CELLS FROM A THIRD PARTY DONOR ON BONE MARROW FAILURE FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: A PILOT STUDY

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Background. Bone marrow failure is a kind of refractory complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). **Aims.** In this study ,we evaluated prospectively the efficiency and safety of expanded mesenchymal stem cells (MSCs) from bone marrow of a third party donor to patients with bone marrow failure after allo- HSCT. **Methods.** Twenty patients with bone marrow failure including primary failure in 7 and secondary failure in 13 received MSCs at a dose of 1×10^6/kg for 1 to 3 cycles, with an interval of 28 to 30 days. **Results** Seventeen patients were responsive to MSCs and 3 were not after 1 to 3 cycles of treatment. Within the first 100 days after MSCs treatment, 13 patients developed 20 episodes of infection. Moreover, 5 patients occurred cytomegalovirus-DNA viremia and 7 occurred Epstein-Barr virus (EBV)-DNA viremia within the first 100 days after MSCs treatment, 3 of whom developed into EBV-associated posttransplant lymphoproliferative disorders (PTLD) in the follow-up period. One patient occurred grade Ⅱ acute graft-versus-host disease (GVHD) and 2 occurred chronic GVHD after receiving the MSCs treatment, including 2 occurred acute GVHD and local chronic GVHD, respectively, after they accepted donor lymphocyte infusion because of PTLD. Following up of 508 (range, 166 to 904) days post-transplants, 9 patients were alive and 11 died. Short-term toxic side effect were not obsvered and 2 patients experienced leukemic relapse after MSCs treatment. Except for 3 episodes of PTLD, other secondary tumor did not occur. **Conclusions** MSCs from bone marrow of a third party donor is effective to patients with bone marrow failure after allo-HSCT, whether it might increase the risk of EBV reactivation and EBV-associated PTLD need further study.

0994

LOW COUNTS OF PLASMACYTOID DENDRITIC CELLS AFTER ENGRAFTMENT ARE ASSOCIATED WITH HIGHER EARLY TRANSPLANTATION-RELATED MORTALITY IN PATIENTS RECEIVING UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The heterogeneous status of host immune defenses may influence the risk of infection and graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation (HSCT). In such defense, dendritic cells (DC), which act as specialized antigen-presenting cells that bridge the innate and adaptive immune systems, are essential cell components. **Aims.** To monitor the recovery of different subsets of DC after unrelated umbilical cord blood (UCB), bone marrow (BM) and peripheral blood (PBSC) HSCT, and to evaluate the impact of the distribution of these cell subsets on the outcome of the transplant. **Methods.** DC [lineage negative, HLA-DR+ and CD123+ plasmacytoid(p)DC, CD11c+ myeloid(m)DC, and CD16+ monocytoid(mo)DC] were quantified by multiparametric flow cytometry at 6 sequential time points (at engraftment, and at days 3, 7, 14, 21 and 60 after engraftment). Overall, 34 patients (19 male; median age 13y, range 1-63y) receiving a UCB (n=15), BM (n=14) or PBSC (n=5)unrelated HSCT were studied. The most common diagnosis was acute leukemia (ALL, 12 cases; AML, 10; CML, 5; aplastic anemia/MDS, 6; Hodgkin lymphoma, 1; SCID, 1). Most patients received myeloablative conditioning (MAC) regimens (73%). Antithymocyte globulin (ATG) was used in 38% and total body irradiation (TBI) in 41% of cases. Median time to neutrophil engraftment was 18 days (range: 12-45). Median follow up time was 6 months. **Results.** Patients who died from early transplantation-related causes (TRM) had significantly lower counts of pDC and mDC during the first 3 weeks after HSCT. At day 21 after engraftment, the median number of pDC and mDC was 0.9 and 2.0/uL among patients who died from TRM vs. 7.1 (p=.006) and 8.4/uL (p=.01) in the remainder, respectively. Patients presenting grade II-IV acute GVHD also had significantly lower pDC counts at days 14 and 21. There was no significant association of both the hematopoietic stem cell source and the conditioning regimen on the risk of TRM or acute GVHD. **Conclusions:** Low pDC counts in the first weeks after unrelated HSCT are associated with an increased incidence of GVHD and mortality. The precise mechanisms that might explain the role of pDC on immunity early after HSCT deserve further investigations.

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PROGNOSTIC IMPACT OF MIXED CHIMERISM AND MINIMAL RESIDUAL DISEASE IN MDS/AML AFTER ALLOGENEIC STEM CELL TRANSPLANT

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Background. delayed full donor T cell quimerism may be associated with a high incidence of relapse in the context of reduced intensity conditioning (RIC) and T-cell depletion (TCD) in myeloid malignancies. The impact of T cell mixed chimerism (MC) has not been studied in myeloablative conditioning, T-cell repleted transplants, nor the relationship with minimal residual disease (MRD).**Aims.** To identify factors associated with (MC) and its impact MDS/AML evolution after transplant. **Methods.** 99 patients (40/59 female/male, 52±12 years) with MDS (n=47)/AML (n=52), allotransplanted (84% RIC) in two different units from Jan-2005 to Dec-2011, were studied. Demographic, clinical and analytical data (including chimerism and minimal residual disease at day 100) were collected. Univariant comparisons between patients with and without MC were done using T or Chi-square tests as appropriate. Variables with a p<0.1 were included in a logistic regression analysis, and odds ratios (OR [95% confidence interval]) were calculated. Patients with and without relapse were also compared using the same methodology. Survival was studied using the Kaplan-Meier method and the log rank test. A p<0.05 was considered significant in multivariate analysis.**Results.** Status at transplant was complete remission in 64%, refractory/progressive disease in 6% and others in 30%. TCD was part of the GVHD prophylaxis in 16%, Tacrolimus-Rapamicin in 14%, all others were CyclosporinA based. Mixed T cell chimerism was present in 57 patients at day +100. Factors associated with MC at day 100 in univariate análisis were sex (MC in male 69% vs 45% in females, p=0.015), infused CD34 cells (5.6±2.16 vs 4.7±2.2 $x10^6$ cells/Kg in those with or without mc respectively, p=0.049) and acute GVHD (43% vs 68% of MC in patients with and without acute GVHD respectively, p=0.014). In multivariate analysis, male sex (OR 2.97 [1.21-7.3]) and aGVHD (OR 0.29 [0.12-0.74]) were significantly related to development of MC. After a median follow up of 2 years overall and relapse free survival were 57 and 60%,. Factors related to relapse in univariant analysis were age (47±13 vs 53±11 years for patients with and without relapse, p=0.021), chronic GVHD (15% vs 41% of relapse in those with or without chronic GVHD, p=0.004), MC at day 100 (32% of relapse vs 10% in patients with complete chimerism, p=0.01), positive MRD at day 100 (50% of relapse vs 14% in cases of negative MRD, p=0.003) and mieloablative conditioning (44% of relapse vs 19% in cases of RIC, p=0.03). In logistic regression analysis, only presence of MC (OR 10.1 [1.6-64]) and positive MRD at day 100 (OR 5.7 [1.1-29.9] remained significant. Based on these results, the sample was stratified into four groups according to chimerism and MRD. Relapse rates (RR) among these groups were significantly different (log-rank p<0.01): Group 1 (MRD neg, CQ: RR 4%), group 2 (MRD neg, MC: RR 23%), group 3 (MRD pos, CQ: RR 25%), group 4 (MRD pos, MC: RR 60%).**Conclusions**: Evaluation of MRD plus T-cell chimerism at day 100 may help to identify patients at high risk for relapse in AML/MDS after allotransplantation.

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FLUDARABINE + EXPOSURE-TARGETED BUSULFAN IN CHILDREN WITH MALIGNANT AND NON-MALIGNANT DISEASES: AN EFFECTIVE AND LOW TOXIC REGIMEN

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Background. Busulfan (Bu) as myeloablative agent is used in conditioning regimens prior to HSCT. We recently found a clear association between Bu-exposure and outcomes. Comparison studies in adults showed a favourable toxicity profile for fludarabine+busulfan (FludBu) compared to the conventional BuCy regimen.**Aims.** We recently initiated a prospective study analysing the effectively of FluBu in myeloid malignancies and all non-malignant indications in pediatrics. We compared the outcomes with our Bu(-exposure targeted)/Cy/(Mel) from a previous cohort (2005-2008). **Methods.** Fludarabine 40mg/m2 was given in 1 hour prior to a 3 hour infusion of once daily busulfan. The target area under the curve (AUC) for Bu was 75-95 mg*h/L (in total) in both groups. Bu dose targeting, based on therapeutic drug monitoring was performed before the second dose. Primary endpoint was event free survival (EFS) and survival. Secondary endpoints were acute graft-versus-host disease (aGvHD), VOD, IPS, neutropenic period and the number of erythrocytes and thrombocytes transfusions. A risk factor analysis was performed using univariable and multivariable COX regression.**Results.** 103 patients were included: 65 unrelated-CBT, 21 MSD and 17 MUD. 55 patients were included in the FludBu group (median follow up 11 mths; range 1.1 - 36) and 48 in the BuCy(Mel) group (44 mths; range 0.2 - 81). The median exposure of Bu was 88 (81-100) mg*h/L in FluBu and 82 (74-104) mg*h/L in BuCy(Mel). The groups were comparable regarding age, cell source, gender, indication for BMT and match-grade. The probability on EFS in FludBu and BuCy(Mel) was 76+/-6% and. 65+/-7% (NS), resp. No difference in aGvHD (≥grade 2: 27 vs.26%) was found. Less IPS was seen after FluBu 3 +/-3% vs. 31+/-7% (p=0,001) and less VOD was seen in BuFlu 3% vs. 22% (p=0.01).A trend to a lower "non-relapse mortality"was found in the FluBu group 7+/-4% vs. 19+/-6% (p=0.08). The period of neutropenia was median 11 in the FludBu compared to 20.5 in BuCy(Mel) (HR 0.38, p=0.05, CI95% 0.20-0.75). The median number of erythrocytes transfusion was 1 (range 1-13) in the FludBu group and 5 (0-22) in the BuCy(Mel) group (p=0.02) and thrombocyte transfusions 4 (range 0-33) vs 10 (range 2-44; p=0,02).**Conclusion:** Bu with a total target AUC of 75-95mg*h/l in combination with Flud showed to be an effective and lower toxic regimen in comparison to BuCy(Mel). A shorter neutropenic period and a lower number of transfusions were needed in FludBu. FludBu as reduced toxicity regimen showed promising results.

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DISPARATE CD52 EXPRESSION RESULTS IN DIFFERENTIAL OUTGROWTH OF LYMPHOCYTE SUBSETS EARLY AFTER LOW DOSE ALEMTUZUMAB-BASED T CELL DEPLETION EXPLAINING LOW INCIDENCE OF EARLY VIRAL COMPLICATIONS

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Despite profound T-cell depletion by in vitro (20 mg) and low dose in vivo (30 mg) Alemtuzumab (ALT, anti-CD52) for acute GvHD prevention, no early CMV disease and a low incidence (2%) of EBV-associated PTLD was seen in a cohort of 60 patients receiving allogeneic stem cell transplantation (alloSCT) after reduced intensity, fludarabin and busulphan-based conditioning.We hypothesized that antiviral protection in the presence of circulating Alemtuzumab early after alloSCT is based on survival of protective memory and effector cells and preferential deletion of naïve T-cells capable of inducing acute GVHD.Since significant Alemtuzumab levels are detected up to 9 weeks after alloSCT, we analyzed the recovery and CD52 expression of NK-cells and CD4 and CD8-positive T-cell subsets (including CMV- and EBV-specific T-cells) during the first 9 weeks after alloSCT.Three weeks after alloSCT, already 70% of the patients had normal levels of circulating NK-cells (median 71x10^6/L, range 0-441x10^6/L). This percentage increased to 89% after 9 weeks (median 217x10^6/L, range 1-1424x10^6/L). After 9 weeks, 30% of the patients had normal numbers of circulating CD8 positive T-cells (median 108x10^6/L, range 1-1539x10^6/L) and only 6% had normal numbers of circulating CD4 positive T-cells (median 88x10^6/L, range 2-612x10^6/L). Interestingly, naive (CD27+CD45RA+) T-cells were virtually absent in the first three months after alloSCT, explaining the low incidence of acute GvHD. A significant proportion of T-cells showed loss of CD52 expression due to GPI-anchor protein deficiency. Using tetramer staining and flowcytometric cell sorting, CD52 positive and CD52 negative CMV and EBV-specific CD8 T-cells were isolated and cytotoxicity and cytokine production assays revealed proper functionality of both the CD52 positive and CD52 negative cells, demonstrating their anti-viral protective capacity. Since NK-cells and a significant proportion of the CD8 positive T-cells surviving in the presence of detectable levels of circulating ALT (median levels 0.39, 0.05 and 0.02 ug/ml, after 3, 6 and 9 weeks, respectively) were CD52 positive,,we examined CD52 expression levels and in-vitro sensitivity to ALT-mediated complement-dependent cytotoxicity (CDC) of NK-cells and CD4 and CD8 positive T-cells in healthy control individuals. CD52 expression was highest on CD4 positive T-cells. Compared to CD4 positive T-cells, the relative CD52 expression on CD8 positive T-cells and NK-cells was only 58% (range 51-70% , n=5) and 27% (range 14-39%, n=5), respectively, resulting in relative protection of NK-cells and CD8 positive T-cells against ALT-mediated CDC, explaining their early outgrowth directly after transplantation. Within CD8 positive T-cells, CD52 expression was highest in the naïve compartment, whereas the relative CD52 expression in antigen-experienced CD8 positive T-cells was 80% (range 76-86%) compared to naïve cells.In conclusion, after ALT-based T-cell depletion, early outgrowth of NK-cells and CD8 positive T-cells in the presence of circulating Alemtuzumab levels is enabled by their low membrane expression of CD52 compared to CD4 positive T-cells and B-cells. Naïve CD8 positive T-cells show a relatively high CD52 expression explaining their prolonged absence after allo-SCT. This resulted in a low incidence of viral complications in spite of T-cell depletion for GVHD prevention.

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RETROSPECTIVE ANALYSIS OF STEM CELL TRANSPLANTATION FROM AN HLA-MISMATCHED DONOR AND IMPACT OF PROPHYLAXIS FOR GRAFT-VERSUS-HOST DISEASE

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Background. GVHD is a major morbidity in patients who have received allogeneic stem cell transplantation (allo-SCT). Although it has been established that the incidence and the severity of acute GVHD (AGVHD) are increased in patients receiving allo-SCT from an HLA-mismatched donor, the impact of GVHD prophylaxis on severity of GVHD in patients who have undergone HLA-mismatched SCT is not known. **Aims.** To evaluate the clinical impact of HLA-mismatched SCT and prophylaxis for GVHD on the incidence and severity of GVHD, transplantation-related mortality (TRM) and survival, we conducted a retrospective analysis in Hokkaido, Japan. **Methods.** Clinical data were obtained for 393 patients who met the following criteria: diagnosed between 2000 and 2009, first time for allo-SCT, bone marrow (BM) or peripheral blood stem cells (PBSC) as a stem cell source, and aged more than 15 years.Results. The median age of the patients was 44 years (range: 15-73 years). The diagnoses were AML (n=131), ALL (n=69), Lymphoma (n=68), MDS (n=48), CML (n=43) and other diseases (n=34), with 38% of the patients being at high risk and 62% of the patients being at standard risk. Thirty-four percent of the patients underwent SCT from a genotypically HLA-matched related donor (MRD), 34% of the patients underwent SCT from a genotypically HLA-matched unrelated donor (MUD) and 32% of the patients underwent SCT from a genotypically HLA-mismatched donor (MMD). Sixty-one percent of the patients received myeloablative conditioning and 39% of the patients received reduced-intensity conditioning. Seventy-eight percent of the patients received BM and 22% of the patients received PBSC. Forty-six percent of the patients received cyclosporine A and short-term methotrexete (CSP+sMTX), and 51% of the patients received tacrolimus (TK) +sMTX as a GVHD prophylaxis. CSP and TK were continuously administered from day -1 at starting doses of 2-3 mg/kg and 0.02-0.03 mg/kg, respectively. MTX was administered intravenously on days 1, 3 and 6, and the doses were 15 mg/m2, 10 mg/m2 and 10 mg/m2, respectively. TK+sMTX was administered more frequently in the patients who underwent SCT from MUD or MMD (P<0.001). AGVHD, grade II-IV AGVHD and grade III-IV AGVHD occurred in 60%, 40% and 17% evaluable patients, respectively, at median onset day of 26. Chronic GVHD occurred in 51.4% evaluable patients. After a median follow-up period of 41 months, 5-year overall survival rate (OS) was 50.8% and there was no difference between the donor groups (P=0.70). TRM was significantly increased in patients who underwent SCT from an MMD (P=0.03), and grade III-IV AGVHD was marginally significant (P=0.06). In patients who developed grade III-IV AGVHD, OS was significantly decreased (5y-OS: 57.4% vs. 25.6%, P<0.001). Among patients who received HLA-mismatched SCT, incidence of grade III-IV AGVHD was significantly lower in patients who received TK+sMTX than in patients who received CSP+sMTX (P=0.02). OS and TRM were not different between the GVHD prophylaxis groups of the patients who underwent HLA-mismatched SCT (P=0.59 for OS and P=0.24 for TRM).Conclusions: TK+sMTX as a GVHD prophylaxis decreased severe AGVHD in patients who underwent HLA-mismatched SCTs.

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EPSTEIN-BARR VIRUS ASSOCIATED CENTRAL NERVOUS SYSTEM DISEASES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Central nervous system(CNS) viral infections, especially Epstein-Barr virus(EBV) infection, are not rare in recipients of transplants.**Aims.** To evaluate CNS infection of EBV in the recipients of allogeneic hematopoietic stem cell transplantation(allo-HSCT). **Methods.** Fifty-three patients were enrolled in this prospective study, including 34 patients with EBV-associated diseases, 11 with asymptomatic EBV-DNA-emia and 8 with CNS manifestations but without EBV-DNA-emia. The levels of EBV-DNA in cerebrospinal fluid(CSF) were detected by real-time polymerase chain reaction assay(RQ-PCR). Otherwise, cytomegalovirus (CMV) - and BK virus (BKV)-DNA were detected by RQ-PCR, and herpes simplex virus (HSV) types 1 and 2-, varicella zoster virus (VZV)- and human herpes virus types 6-8 (HHV6-8)- DNA were detected by qualitative PCR in blood and CSF. **Results** Of the 45 patients with EBV-DNA-emia, 12 patients were EBV-DNA positive, 1 was CMV-DNA positive and 1 HSV-1-DNA positive, respectively, in CSF. In 8 patients with CNS manifestations but without EBV-DNA-emia, EBV-, HSV-1- and VZV-DNA were detectable in 1, 2 and 1 case, respectively, in CSF. All patients who were virus-DNA positive in CSF had CNS manifestations. These patients all were simultaneous corresponding viremia except 1 patient, who had EBV-DNA-emia at 19 days after the onset of EBV-DNA positivity in CSF. Magnetic resonance imaging (MRI) revealed that 10 cases were abnormal and 8 normal in the 18 patients who were virus-DNA positive in CSF. Thirteen patients were diagnosed as EBV-associated CNS diseases, 3 as HSV-1 encephalitis, 1 as CMV encephalitis and 1 VZV encephalitis according to the results of CSF, MRI and so on. Of the 13 patients with EBV-associated CNS diseases, 7 cases were diagnosed as EBV-associated encephalitis/myelitis and 6 as post transplant lymphoproliferative disorder(PTLD) at the time of disease onset. With the development of disease, 1 case who was diagnosed as encephalitis initially had space-occupying lesions in CNS, and diagnosed as PTLD finally. The time of EBV-associated CNS diseases onset was from +22 days to +184 days (median time, +46 days) post-transplantation. The patient with CMV encephalitis occurred on days +41, 1 VZV on days +308, and 3 HSV-1 on days +29, +938 and +1245 post-transplantation, respectively. The EBV-DNA levels of CSF were higher than that of blood in patients with EBV-associated CNS diseases. The EBV-DNA levels of blood and CSF declined with control of the diseases. On the contrary, the EBV-DNA levels of CSF increased, and blood increased or remained unchanged with the development of the diseases in those who were unresponsive to the treatment. To now, 9 patients were alive (EBV-associated CNS diseases in 6, HSV-1 encephalitis in 2 and VZV encephalitis in 1, respectively) and 9 death.**Conclusions** EBV infection in CNS is common, as well as more frequently than other opportunistic viral infections in the early times of allo-HSCT. Both encephalitis/myelitis and PTLD may act as the initial presenting manifestation, and encephalitis/myelitis can progress to CNS PTLD with the development of disease. A few EBV-associated CNS diseases might be EBV-DNA negative of blood at the time of disease onset.

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EPSTEIN-BARR VIRUS LOAD IN CEREBROSPINAL FLUID AND PERIPHERAL BLOOD OF PATIENTS WITH EBV-ASSOCIATED CENTRAL NERVOUS SYSTEM DISEASES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The EBV-DNA loads of blood could act as an indicator for development and therapeutic evaluation of EBV-associated diseases other than central nervous system (CNS), but whether it also could act as an indicator for development and therapeutic evaluation of EBV-associated CNS diseases need to be defined. **Aims.** To evaluate the diagnostic and prognostic utility of monitoring the Epstein-Barr virus (EBV) load in the cerebrospinal fluid (CSF) and peripheral blood for the patients with EBV-associated central nervous system (CNS) diseases after allogeneic hematopoietic stem cell transplantation (allo-HSCT). **Methods.** 172 patients undergoing allo-HSCT were enrolled in the prospective study.The EBV-DNA levels of blood were monitored regularly with RQ-PCR in recipients of transplants for 3 years post-transplantation. The EBV-DNA levels of CSF were monitored in patients with EBV-associated CNS diseases before the treatment and at different points following the treatment. **Results** Twenty-seven patients occurred post-transplant EBV-associated diseases, including 12 patients with EBV-associated CNS diseases. The 3-year cumulative incidences of EBV-associated diseases and EBV-associated CNS diseases were 19.5 ± 3.5% and 8.6 ± 2.4%, respectively. Patients with EBV-associated diseases showed higher loads of EBV-DNA in their blood than that of patients with EBV-DNA-emia. There was no difference between the EBV-DNA levels of blood in patients with CNS involvement and patients without CNS involvement. The EBV-DNA loads of blood increased 3 to 14 days before the clinical manifestations of EBV-associated diseases emerged. The EBV-DNA loads of CSF were higher than that of blood in patients with EBV-associated CNS diseases. In 12 patients with EBV-associated CNS diseases, EBV-DNA levels were declining both in blood and CSF with the control of diseases and the EBV-DNA loads of CSF decreased faster than that of blood in 5 patients who responded to treatment, and the EBV-DNA levels of CSF increased in 5 patients who were unresponsive to treatment. Otherwise, in 2 patients, their EBV-DNA levels in blood decreased and lymphadenectasis became smaller after the use of intravenous rituximab-based treatment, but their EBV-DNA levels in CSF increased and CNS manifestations did not have improvement or worsened. And their EBV-DNA levels in CSF quickly decreased and CNS manifestations were improved after they treated with intrathecal rituximab. On multivariate analysis, the use of antithymocyte globulin and intensified conditioning regimens were independent risk factors for EBV-associated diseases and EBV-associated CNS diseases. **Conclusions.** EBV-associated CNS diseases are not rare after allo-HSCT. The EBV-DNA loads of CSF could act as an important indicator, but the EBV-DNA loads of blood could not, for the diagnosis, prognosis and therapeutic evaluation of EBV-associated CNS diseases.

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THE SUCCESSFUL TRANSPLANTATION OF SEVERE APLASTIC ANEMIA WITH AUTOLOGOUS CORD BLOOD

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Background. Cord blood transplantation may be curative for patients with acquired and genetic disorders of hematopoiesis and use of cord blood as an alternative hematopoietic stem cell source has increased substantially. At present, cord blood transplantation has been limited to patients who do not have a matched family or unrelated donor. We report the successful autologous cord blood transplantation in the treatment of two children with severe aplastic anemia. **Methods.** Two patients with severe aplastic anemia whose cord blood units had been collected at birth and stored for years prior to onset of pancytopenia were treated with autologous cord blood transplantation: one in a 6-year old boy and the other in a 3-year boy. The preparative regimen consisted of cyclophosphamide 50mg/kg/d on days -7 through -4, rabbit antithymocyte globulin(Thymoglobulin) 2.5mg/kg/d on days-6 through -3, followed by infusion of 1.8×10^7 , 6.0×10^7 total nucleated cells per kilogram containing 1.28×10^5 ,0.9×10^5 $CD34^+$ cells per kilogram.Results. White blood cell engraftment (ANC $>0.5 \times 10^9$/L) occurred by day 18, 29 and platelet engraftment ($> 20 \times 10^9$/L) by day 31, 46. The patient's recovery was unremarkable. At 1 year and 6 years of follow-up, the patients are in complete hematologic remission with a normal complete blood count. **Summary**. We report the two cases of successful autologous cord bloodtransplantation for the treatment of severe aplastic anemia.

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A WEB-BASED STEPPED CARE INTERVENTION FOR PSYCHOLOGICAL DISTRESS AND QUALITY OF LIFE AFTER AUTO-SCT FOR HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CLINICAL TRIAL

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Background. In the first year after treatment with auto-SCT, anxiety and depressive symptoms are being reported by approximately 18% and 26-36% of the patients, respectively. Psychological distress has shown to be a strong predictor of health-related quality of life in cancer patients following stem cell transplantation. Psychological treatment is hypothesized to improve psychological distress, functional status and other aspects of quality of life in these patients. We offer patients a stepped care intervention, including internet self-help therapy, in a randomized clinical trial.**Aim.** The aim is to evaluate the outcome of a stepped care intervention for psychological distress on functional status and other aspects of quality of life in patients with hematological malignan-

cies treated with auto-SCT. **Methods.** The study is designed as a randomized clinical trial with 2 treatment arms: stepped care versus care as usual. Stepped care consists of (1) watchful waiting; (2) internet self-management program; (3) face-to-face counseling, medication, or referral. **Results.** The study started in August 2009 and is still ongoing. Preliminary analyses show that just before transplantation, 51% of the patients are psychologically distressed. At 13, 30 and 42 post-transplant, this percentage rises from 30 to 43%, with female patients being more likely to be distressed. Up to now, 29 patients were offered treatment with the online self-help intervention 'Stress under control', and no patients were referred to step 3 of the stepped care program. Of the 21 patients who chose to start with online self-management therapy, 12 quit after lesson 1 or 2. The other 9 patients completed the intervention successfully; preliminary evaluation showed a reduction of psychological distress in this group.**Summary and Conclusions.** This study has several innovative characteristics. First, the outcome of the intervention for psychological distress in auto-SCT patients is evaluated in a randomized clinical study. Furthermore, the intervention concerns an web-based treatment in the first step. Finally, the intervention is characterized by an emphasis on self-management, efficiency, and a multi-disciplinary approach. In the treatment of psychological distress, internet therapy seems to be a valuable option for hematological cancer patients undergoing auto-SCT. However, not all patients judge this treatment type to be helpful. Detailed assessment of the need for psychological treatment, in a face-to-face contact, seems to be important in this intensively treated patient group. Patients' need for treatment should be assessed, and intensity of treatment should be tailored to the specific needs of the individual patient.

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QUALITY OF LIFE IN BLOOD CANCER PATIENTS IN COMPLETE REMISSION AT LONG-TERM FOLLOW-UP AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT)

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It is now clearly established that cure or control of the underlying malignancy is not necessarily accompanied by full restoration of health or quality of life (QoL) in long-term survivors. At the same time, there is lack of information about QoL profile in blood cancer patients in complete remission at long-term follow-up after ASCT. We aimed to examine QoL in patients with hematological malignancies in complete remission at long-term follow-up after AHSCT as compared with population norms.A totalof 72 patients with hematological malignances (non-Hodgkin lymphomas - 24 patients, Hodgkin lymphoma - 24, multiple myeloma - 20, others - 4) in complete remission with the follow-up period of at least 12 months were enrolled in the study (mean age 40.3, SD-12.7; male/female -33/39). All patients were treated by AHSCT. Mean follow-up was 24 months. QoL was assessed using generic questionnaire SF-36. To compare patient population with normative data the sample from population norm (PN) data base adjusted to age and gender (n=72) was used. For comparisons t-test for independent samples or Mann-Whitney test was used. Distribution of patients according to the grades of QoL impairment was analyzed: no QoL impairment (Integral QoL index similar to the one in normative population sample), mild (25% decrease from a PN), moderate (25-50% decrease), severe (50-75% decrease), and critical(>75% decrease).QoL parameters in patients in long-term remission were similar to the ones in general population for the majority of scales: role-physical functioning - 75.0 vs 78.6, bodily pain - 85.9 vs 79.7, general health - 60.5 vs 66.1, vitality - 66.9 vs 68.9, social functioning - 84.6 vs 77.4, role-emotional functioning - 79.5 vs 74.1, and mental health - 72.0 vs 66.4. The value of physicalfunctioning scale was significantly lower in patients as compared to PN: 77.1 vs 92.4 ($p<0.001$). The majority of patients had no QoL impairment (55 patients); 5 patients - mild QoL impairment, 7 - moderate QoL impairment; 4 - severe QoL impairment, and 1 - critical QoL impairment.In conclusion, patients with hematological malignances in complete remission with follow-up of at least 1 year after ASHT had QoL parameters similar to normative data except physical functioning. The vast majority of patients exhibited either no or mild to moderate QoL impairment. At the same time, 7% of patients had either severe or critical QoL impairment. Further studies are needed to examine QoL profile in this patient population to better define treatment outcomes after AHSCT.

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A DEDICATED GVHD CLINIC CAN IMPROVE THE QUALITY OF LIFE FOR CANCER SURVIVORS

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Background. Graft versus host disease (GVHD) remains a major cause of morbidity and mortality following haematopoietic stem cell transplantation (SCT). These cancer survivors can have complex medical, psychological, physical and social issues which may not be fully addressed in standard transplant follow-up clinics due to time restraints or lack of resources. A specialist GVHD clinic was established at the Royal Marsden NHS Foundation Trust,UKto try and improve the quality of care for these patients.AimsTo evaluate the impact of the new GVHD clinic on quality of life, morbidity and mortality and referral patterns. **Methods.** All patients who attended the clinic from 1/9/2010 to 1/9/2011 were included in the study. Data were collected from the electronic patient record. A proforma was completed for each new patient and patients were asked to complete FACT-BMT and chronic GVHD symptom scale questionnaires as a measure of health related quality of life. The study was approved by the Royal Marsden NHS Foundation Trust audit committee. **Results**. Thirty patients attended the clinic (24 had chronic GVHD and 6 had acute GVHD). 20 patients were male and the median age was 48 years (range 22-68 years).Ten patients had severe chronic GVHD and 14 had moderate GVHD according to NIH criteria. All patients with acute GVHD had grade III/IV disease. 26/30 (87%) were within one year of transplant. 24/30 (80%) were on two immunosuppressive agents. 17/30 (57%) had single organ involvement, 13% had two organs involved and 30% had 3 or more organs involved. 42 referrals to other specialists were made. The most frequent referrals were made to dermatology (13/30) and ophthalmology (8/30). Patients frequently required input from the dietician (12/30), physiotherapist (8/30), occupational therapist (5/30) or psychological medicine (8/30). Ten patients stopped immunosuppression and 13 remained on immunosuppression at last follow up. Seven patients died (2 GVHD alone, 5 GVHD plus infection). A subset of patients completed the chronic GVHD symptom scale and FACT BMT questionnaires as a measure of quality of life. The patients with more severe GVHD according to NIH criteria had lower chronic GVHD symptom scores and higher FACT BMT scores indicating poorer quality of life. Nine patients completed the cGVHD symptom scale at different time points. All patients showed an improvement in scores and, in five patients, this improvement was large enough to suggest a significant change in quality of life. **Conclusions.** This pilot study evaluated the first year of a new dedicated GVHD clinic. This report shows that the clinic led to active involvement of the multidisciplinary team, referrals to specialists with an interest in GVHD and preliminary data suggest an improvement in patients' quality of life.

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BIOCHEMICAL MARKERS FOR EARLY DETECTION AND PREDICTION OF CARDIOTOXICITY IN PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Cardiac events are emerging life-threatening complications after allogeneic hematopoietic stem cell transplantation (HSCT). **Aims.** We aimed to *assess the predictive* value of high sensitive cardiac troponin T (hs-cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) testing and early identification of patients at high risk of a cardiac event after HSCT. **Methods.** Sixty-three patients with the median age of 37 years at the time of allogeneic HSCT for hematologic diseases were studied. Fifty patients were conditioned with myeloablative regimens and the remaining 13 patients with nonmyeloablative regimens. Forty-four patients (69,8%) were previously treated with anthracyclines. Cardiac biomarkers were serially measured before conditioning regimen and at days 1, 14 and 30 after HSCT. Cardiac systolic and diastolic functions were assessed before conditioning regimen and 1 month after HSCT by echocardiography. Written informed consent was obtained from all patients. **Results.** The differences in plasma NT-proBNP and hs-cTnT concentrations during the 30 days following the HSCT were statistically significant ($P < 0,01$ v.s. $P<0,01$). Thirty days after HSCT, we found a statistically significant decrease in systolic and diastolic parameters performed by echocardiography ($P<0,01$ v.s. $P<0,01$). Seven of 63 patients (11%) developed a cardiac event defined as cardiac dysrhythmias, pericarditis with cardiac tampon-

ade and heart failure. In two patients, the cardiotoxicity was severe and resulting in death. By multivariate analysis, the strongest predictor of cardiac event was an increased level of hs-cTnT and NT-proBNP persisted for a period 14 days after HSCT (P < 0,01). The use of hs-cTnT at day 14 after HSCT (with the diagnostic cutoff value of 0,014 µg/L) had a sensitivity of 100% and a specificity of 83.9%. The use of NT-proBNP (with the diagnostic cutoff value of 1411 pg/mL) at day 14 after HSCT had also a sensitivity of 100% and a specificity of 83.9%. Results of hs-cTnT testing were better than those of NT-proBNP testing, with significantly greater area under the curve (0.93 vs 0.91, P < 0,01). The area under the curve from hs-cTnT testing plus NT-proBNP testing (0.95) was superior to each diagnostic modality alone. **Conclusions**. Receiver-operating characteristic analyses demonstrated hs-cTnT and NT-proBNP to be highly sensitive and specific for the diagnosis of cardiotoxicity after HSCT. Measurements of plasma NT-proBNP and hs-cTnT concentrations might be a useful tool for identification of high-risk patients requiring further cardiological follow up. Measurement of hs-cTnT plus NT-proBNP was superior to hs-cTnT and NT-proBNP measurements alone. Further studies are needed to clarify whether combination of both biomarkers improve detection of cardiotoxicity.This work was supported by a grant from the Scientific Grant Agency of the Ministry of Health, Slovak Republic 2007/42-UK-18.

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COST-EFFECTIVENESS ANALYSIS OF VORICONAZOLE FOR PROPHYLAXIS OF INVASIVE FUNGAL INFECTIONS IN SPANISH PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background. In order to prevent some of the high mortality associated with invasive fungal infections (IFI), antifungal prophylaxis (AFP) may be indicated for allogeneic hematopoietic cell transplant (alloHCT) recipients at high risk for IFI, in particular for invasive mould infections. **Aims.** To evaluate the cost-effectiveness of voriconazole compared with other oral azoles (fluconazole, itraconazole, posaconazole) as primary AFP in patients undergoing alloHCT in Spain. **Methods.** A decision analytic model was used to simulate prophylactic treatment in a hypothetical cohort of 1000 patients undergoing alloHCT. Costs and outcomes for a 180-day period post-transplant were estimated. A previously presented, mixed treatment comparison (MTC) of randomized clinical trials (RCTs) was used to derive the probability of developing invasive aspergillosis (IA), invasive candidiasis (IC), or other IFI, as well as the proportions of patients requiring other licensed antifungal treatment (OLAT). Duration of AFP was derived from the clinical trial data included in the MTC (posaconazole prophylaxis was assumed to have the same duration as voriconazole prophylaxis) and confirmed by expert opinion. Costs of treating breakthrough IFI and IFI case fatality rates were based on published literature. The model considered direct costs associated with drug acquisition (separately for AFP and OLAT), IFI monitoring, and IFI treatment from the perspective of the Spanish national health system in 2011. **Results.** Costs and outcomes based on the hypothetical 1000-patient cohort are summarized in the table. Itraconazole was associated with the lowest total cost (€15,210,908) followed by fluconazole (€17,636,100), voriconazole (€19,443,320), and posaconazole (€23,615,334). Voriconazole was associated with the lowest number of breakthrough IFI cases (n=30), fewest deaths from breakthrough IFI (n=21), and fewest deaths from any cause (n=217). Voriconazole was also associated with the most life-years gained. Compared with estimates for posaconazole, voriconazole resulted in cost savings of €4,172,014, along with 20 fewer IFIs, 8 fewer deaths from IFI, and 6 fewer total deaths. Compared with fluconazole as control, the number of patients needed-to-treat to prevent one IFI was 20 for voriconazole and 33 for both itraconazole and posaconazole; the number of patients needed-to-treat to prevent one additional death was 36 (voriconazole), 59 (itraconazole) and 47 patients (posaconazole). Results of the base-case analysis showed that posaconazole was "dominated"(ie, more costly and resulted in fewer life years and more IFIs) by voriconazole. Similarly, fluconazole was "dominated"by itraconazole. Compared with itraconazole, voriconazole was associated with higher costs but also resulted in more life years and fewer IFIs. **Conclusions**. Among the second-generation azoles, voriconazole was less costly and more effective (reduced mortality, IFI avoided, and life years gained) than posaconazole as primary AFP in alloHCT recipients 180 days post-transplant. In this model, itraconazole appeared to be more cost-effective than second-generation azoles; this result is not surprising, given that itraconazole is available as an inexpensive generic. However, there are important clinical limitations with itraconazole as AFP, such as relatively poor long-term tolerability and lack of an alternative intravenous formulation in some countries.[Note: AFP currently constitutes off-label use for voriconazole.

	Fluconazole	Itraconazole	Posaconazole	Voriconazole	Voriconazole vs. Posaconazole*
Costs per patient, €					
Prophylaxis costs	591	294	8,628	6,850	-1,778
IFI monitoring costs	8,623	8,623	8,623	8,623	0
IFI treatment costs	5,013	3,429	2,956	1,585	-1,371
OLAT costs	3,409	2,666	3,409	2,386	-1,023
Total cost per patient	***17,636***	***15,211***	***23,615***	***19,443***	***-4,172***
Outcomes per 1000 patients, n					
Episodes of IA	60	40	20	20	0
Episodes of IC	10	0	10	10	0
Episodes of other IFI	10	10	20	0	-20
Total IFI	***80***	***50***	***50***	***30***	***-20***
Deaths from IA	46	31	15	15	0
Deaths from IC	6	0	6	6	0
Deaths from other IFI	4	4	8	0	-8
Deaths from all IFI	***56***	***35***	***29***	***21***	***-8***
Deaths from other causes	189	193	194	196	2
Total deaths	***245***	***228***	***223***	***217***	***-6***
Total life years gained	459.6	461.9	462.6	463.4	0.9
ICER, €					
Cost per additional IFI avoided	Dominated by itraconazole	-	Dominated by voriconazole	211, 621 (vs itraconazole)	Voriconazole dominates
Cost per life year gained	Dominated by itraconazole	-	Dominated by voriconazole	2,812,874 (vs itraconazole)	Voriconazole dominates

All numbers shown have been rounded.
*Incremental difference between voriconazole and posaconazole.

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USE OF PERIPHERAL VENOUS ACCESS IN PERIPHERAL BLOOD STEM CELL COLLECTION: A COST ANALYSIS

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Aims. The cost of a stem cell harvest procedure from peripheral blood is high. It is usual practice to place a central venous catheter to perform the aphaeresis, specially when large volumes of blood are processed. However, this technique is not free of complications and requires hospital admission, which incises in the global cost of the aphaeresis procedure. Obtaining stem cells by peripheral venous access (PVA) is feasible and it is performed in an out-patient basis which could minimize the expense without affecting the outcome of the procedure. Our centre has developed a protocol aiming to use PVA. We present the results of this protocol and a cost analysis. **Methods.** In order to assess the efficacy and cost of a PVA, we obtained the records of the aphaeresis procedure performed during the years 2010 to 2011 in our unit. Inclusion criteria were: weight >50 kg and stem cell mobilization with G-CSF alone or G-CSF and plerixafor for autologous stem cell transplantation. Healthy donors were excluded. Expert nurses assessed whether PVA or central line was to be used for the procedure in the baseline visit in the aphaeresis unit. Stem cells were collected by aphaeresis using a COBE-Spectra (Cobe BTC, Lakewood, CO, USA) to obtain a number of CD34+ cells which was considered enough to perform the transplantation depending on the pathology of the patient. The Economics Evaluation Unit of our hospital calculated the global cost of each aphaeresis procedure. **Results.** A total of 105 aphaeresis procedures from the 78 patients were performed (Median±SD 1.36±0.61). PVA was used in 45 (57.7%) patients. All of the patients had received previous chemotherapy. Median age was 58 (range 7 - 78). Diagnosis was Multiple Myeloma (42.3% patients), Non-Hodgkin Lymphoma (32.1%) and others, including acute leukaemia and Hodgkin lymphoma (25.6%). 51 patients (65.4%) underwent a single aphaeresis procedure, whereas 2 patients required 3. The target CD34+ cells dose was obtained in 72 (92.3%). There was no difference in the number of aphaeresis procedures needed. (1.45 in the PVA vs 1.3 in the central line group. p=NS). 2 patients in the PVA group needed a central line placement due to hematoma in the puncture site before a second procedure due to insufficient stem cells collected in the first day of aphaeresis. Global cost for patient with Central line was 2.402,86 € (Personnel cost 236,90 €, Material 1.325,96 €, Admission 840 €). Global cost in the case of use PVA was 1.418,70 € (Personnel cost: 196,70 €, Material: 1.222,90 €). **Conclusions.** Peripheral venous access can be successfully used for stem cell collection in peripheral blood aphaeresis procedures in previously chemotherapy-treated patients. A throughout assessment by expert nurses in the aphaeresis area is fundamental to increase the use of PVA in aphaeresis procedures. The use of PVA allows a median save of 984.16€ per patient

Stem cell transplantation - Experimental

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IMPACT OF MYELOID DERIVED SUPPRESSOR CELLS FREQUENCIES IN G-CSF MOBILIZED UNRELATED DONOR GRAFTS ON THE INCIDENCE OF ACUTE GVHD AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Myeloid-derived suppressor cells (MDSCs) comprise a heterogeneous population of immature myeloid cells with immunosuppressive capabilities. Recent evidence suggests that in mice graft-versus-host disease (GvHD) can be abrogated by *ex-vivo* expanded, bone marrow derived, MDSCs generated in the presence of GM-CSF, G-CSF and IL-13 (Highfill et al. Blood 2010). Whether MDSCs play a role in human allogeneic hematopoietic stem cell transplantation (allo-HSCT) is still unclear. In this scenario, we hypothesized that G-CSF stem cell mobilization may be protective from acute GvHD (aGvHD) in unrelated donor HSCT by increasing MDSC frequencies. **Methods.** G-CSF-mobilized peripheral blood (PB) samples were collected from 40 healthy unrelated donors (median age 34, range 20-43, male/female 31/9) who received G-CSF (Filgrastim) at 10 µg/kg/day for 5 days. Donor selection had been performed according to standard criteria, including molecular typing for HLA-A, -B, -C, DRB1, and DQB1. Donors were 10/10 HLA-matched (MUD) in 20 cases, 9/10 in 13 cases and the remaining 7 cases were mismatched in 2 or more HLA loci (MMUD). Patients (median age 46, range 18-67) received reduced intensity conditioning based on low-dose total body irradiation (TBI 2Gy) (8), Fludarabine-Rabbit Antithymocyte Globulin (rATG) (9) or Thiotepa-rATG (23) followed by unmanipulated allo-HSCT. Diagnosis were lymphomas (29), myelomas (8), acute myeloid leukemia (3). Patients received cyclosporine plus either methotrexate (36) or mycophenolate mofetil (4) for GvHD prophylaxis. As controls, PB samples were collected from 10 healthy adults (median age 30, range 23-40). Informed consent was obtained from all subjects. Cells were characterized using flow cytometry (MACSQuant Analyzer, Miltenyi Biotec, Germany) with Abs against CD3, CD14, CD16, CD19, CD20, CD56, CD11b (Biolegend, San Diego, CA), HLA-DR, CD33 (Miltenyi Biotec, Germany) after Ficoll separation. The frequencies of MDSCs present in the graft were correlated with the clinical characteristics and outcome of the 40 patients. *Results.* A population of MDSCs defined as CD3/CD19/CD56/CD14$^{-/Lo}$ or Lin$^{-/Lo}$, HLA-DR^{-} CD33^{+}CD11b^{+} was easily detected in G-CSF mobilized PB samples. Expansion of MDSCs in the PB of G-CSF-treated unrelated donors was found with respect to steady state control individuals ($p< 0.03$, Mann Whitney-U). Acute GvHD occurred in 16 of 40 patients (40%). There was no significant correlation between the incidence of aGvHD and the degree of HLA incompatibility or the presence of donor-recipient sex mismatches. Neither the conditioning regimens nor the GvHD prophylaxis had effect on risk of aGvHD. Conversely, aGvHD patients received grafts containing significantly lower number of MDSCs when compared to non-aGvHD patients ($p < 0.006$, Mann Whitney-U). The ability of MDSC levels in the graft to predict the occurrence of aGVHD was determined by the receiver operating characteristic (ROC) curve: sensitivity was 100%, specificity was 60%, area under the curve, AUC=0.768.**Conclusions.** G-CSF mobilization significantly increases circulating MDSCs in Matched Unrelated Donors relative to healthy volunteers. A significant correlation between the frequencies of MDSCs present in the graft and the incidence of aGvHD was found. Although these findings should be confirmed in prospective studies, modulating MDSCs in the graft might represent a promising strategy for GVHD prophylaxis.

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MYELOID-DERIVED SUPPRESSOR CELLS CONTRIBUTED TO CONTROL ACUTE GRAFT-VERSUS-HOST DISEASE IN PATIENTS UNDERGOING UNMANIPULATED ALLOGENEIC BLOOD AND MARROW TRANSPLANTATION

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Background. The regulatory role of myeloid-derived suppressor cells (MDSCs) is emerging in immune tolerance induction of animal transplantation models. Previously our team found MDSCs mobilized by G-CSF in allografts positively correlate with low risk of acute graft-versus host disease (aGvHD) in patients undergoing unmanipulated allogeneic blood and marrow transplantation.**Aims.** The aim of this study was to further investigate :(1) the immune reconstitution of MDSCs after allogeneic transplantation ;(2)their roles during the onset and recovery of aGvHD, as well as the potential regulatory network with alloreactive and regulatory lymphocytes. **Methods.** After unmanipulated allogeneic blood and marrow transplantation, patients were monitored for MDSCs in peripheral blood at +7, +15, +30days. Patients with aGvHD were prospectively analyzed for the subsets and cytokine secretion of MDSCs: monocytic MDSCs (CD33^{+}CD11b^{+}CD14^{+}lin^{-}HLA-DR^{-}), granulocytic MDSCs (CD33^{int}CD11b^{+}CD15^{+}lin^{-}HLA-DR^{-}), as well as Th1/Th17, regulatory T cells (Treg), etc. in peripheral blood at the onset of aGvHD, during therapy and post-therapy evaluation. Prophylaxis for aGvHD post transplantation included cyclosporine A(CsA) and short-term methotrexate (MTX) with mycophenolate mofetil (MMF) The first-line therapy for aGvHD mainly included methylprednisolone, FK506; second-line therapy included anti-CD25 monoclonal antibodies.**Results.** MDSCs were undetectable at+7days, gradually increased from +15days, and nearly recovered to the level of healthy donor around +30days in the absence of aGvHD or poor neutrophils engraftment. There are no significant differences between the immune reconstitution of two subgroups. 10 of 18 patients got I-II degree of aGvHD only involving with skin; other 8 patients got II-III degree of aGvHD with mild to moderate gut involvement. In those patients (n=12) responding well to steroid and adjusting CsA/FK506, monocytic MDSCs are found lower (0.05%-0.64% of nucleated cells)at the onset of aGvHD, rapidly increased to the peak(1.81%-6.79%) as the rash spread or diarrhea become severe, then decreased gradually after partial recovery(PR). The sustaining low level of monocytic MDSCs predicted poor response to the first-line therapy, and those patients (n=6) got partial or complete recovery with anti-CD25 antibodies. Granulocytic MDSCs showed similar pattern yet more obviously in patients involved with gut. The secretion of IL-10 and TGF-beta increased corresponding to the number of MDSCs. The percentage of INF-gamma and/or IL-17 producing CD4+ T cells (Th1/17, etc.) negatively correlated with the variation of MDSCs; while MDSCs slightly increased after the application of anti-CD25 antibodies.**Conclusions**: These data indicate that besides MDSCs in allografts, the immune reconstitution of MDSCs after allogeneic transplantation may also contribute to the control of aGvHD; the expansion of MDSCs in vivo seems to be kind of negative feedback to the alloreactive lymphocytes and aGvHD. Such a regulatory phenomenon may be important to interpret the roles of MDSCs in allogeneic hematopoietic stem cell transplantation and provide base for clinical practice, such as infusion of MDSCs or application of M-CSF/G-CSF post transplantation for prophylaxis and treatment of aGvHD.

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HLA CLASS II RESTRICTED ANTIGENS WHICH PRESENTATION IS ABOLISHED BY HLA-DM AND RESTORED BY HLA-DO ARE APPROPRIATE TARGETS FOR SELECTIVE GRAFT-VERSUS-LEUKEMIA REACTIVITY

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Background. In contrast to the majority of solid organs, hematological malignancies often express HLA class II at their cell surface. HLA class II restricted antigens may therefore be attractive targets for CD4+ T-cells in allogeneic hematopoietic stem cell transplantation (alloSCT) to induce beneficial Graft-versus-Leukemia (GvL) reactivity without Graft-versus-Host Disease (GvHD). In the presence of pro-inflammatory cytokines, however, non-hematopoietic tissues may express HLA class II and become targets for CD4+ T-cells in GvHD. We previously identified various HLA class II restricted antigens as targets for CD4+ T-cells in alloSCT, and demonstrated that these antigens can be divided into DM-resistant and DM-sensitive antigens based on their behavior towards HLA-DM and its natural inhibitor DO. In a human cell line model, pres-

entation of DM-sensitive antigens was shown to be abolished by HLA-DM and could only be restored by HLA-DO. As expression of HLA-DO in its full heterodimer configuration is hardly induced by pro-inflammatory cytokines in non-hematopoietic cells, DM-sensitive antigens are not efficiently presented on non-hematopoietic tissues. DM-sensitive antigens may therefore be ideal targets for CD4+ T-cell based therapies with the aim to induce a selective GvL effect.**Aims**Aim of this study is to investigate whether DM-sensitive antigens are appropriate targets for CD4+ T-cells in GvL reactivity.**Methods**Gene expression of HLA-DRA, invariant chain, HLA-DMA and DMB, HLA-DOA and DOB was measured in primary leukemic cells of different origins by microarray gene expression analysis. T-cell recognition of primary leukemic cells was measured by IFN-g ELISA. HLA-DO expression levels were modified in acute leukemia cell lines by gene introduction and silencing by specific short hairpin RNA.**Results**All HLA class II positive primary leukemic samples were shown to express similar mRNA levels of invariant chain, HLA-DMA and DMB. Also HLA-DOA was highly expressed by all HLA class II positive primary leukemic samples, with exception of a small subgroup of AML samples. HLA-DOB expression was low but present in all AML samples and high in all CLL and some ALL samples. HLA-DOB gene expression in these CLL and ALL samples was comparable to mature DC and B-cells. In the AML subgroup, we could show that lack of T-cell recognition of DM-sensitive antigens correlated with low expression of HLA-DOA. The relevance of HLA-DOA expression was confirmed in ALL cell lines showing that T-cell recognition of DM-sensitive antigens was inhibited upon introduction of HLA-DO specific short hairpin RNA. The experiments furthermore demonstrated and confirmed that T-cell recognition of DM-sensitive antigens was significantly enhanced by HLA-DOB gene overexpression.**Summary/Conclusions**This study shows that the majority of primary leukemic cells express sufficient amounts of HLA-DO to present DM-sensitive antigens, and that expression levels of both components of the HLA-DO dimer are critical for T-cell recognition. The experiments furthermore demonstrate that HLA-DOA and DOB expression in CLL and some ALL cells was comparable to professional antigen presenting cells, suggesting that DM-sensitive antigens may be particularly relevant targets for CD4+ T-cell therapy of leukemic cells of B-cell origin.

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PENTRAXIN-3 INCREASES AT THE ONSET OF GRAFT-VERSUS-HOST DISEASE IN TRANSPLANTED PATIENTS

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Background. Graft-versus-Host Disease (GvHD) is a major obstacle to safe allogeneic haematopoietic stem cell transplantation (HSCT). GvHD pathophysiology is still poorly understood and its diagnosis depends on clinical manifestations and invasive biopsies. Specific biomarkers would facilitate the early and accurate recognition and the monitoring of this invalidating disease. The long pentraxin-3 (PTX-3) is locally produced at sites of inflammation. Its rapid increase in different human inflammatory diseases and the correlation between PTX-3 levels and disease severity suggest the potential usage of this molecule as GvHD diagnostic marker.**Aims.** Our aim was to correlate PTX-3 plasma levels with GvHD occurrence in HSCT patients, to define its potential use as early marker of pathology. **Methods.** Having obtained an informed consent, we collected plasma samples from 92 adults and pediatric patients, who received HSCT, and from 17 healthy donors (HD) volunteers. After HSCT, 56/92 patients developed acute GvHD, while 36/46 never experienced it. Concerning GvHD patients, blood samples were collected at the day of GvHD onset, before the beginning of GvHD-specific drug therapy. PTX-3 plasma levels were monitored by ELISA assays.**Results.** Patients who did not develop GvHD after HSCT showed augmented PTX-3 plasma levels (mean=5.57 ng/ml, range=1.1-22.3 ng/ml) if compared to HD (mean=2 ng/ml, range=0.3-5.4 ng/ml, p<0.01). Interestingly, we observed a strong increase of PTX-3 plasma levels in patients with acute GvHD in correspondence to the disease onset (mean=63 ng/ml, range=6-847 ng/ml, p<0.01 vs no GvHD patients). Since PTX-3 values could be influenced by the occurrence of opportunistic infections, we refined our analysis. In particular, we compared PTX-3 plasma levels measured at the onset of GvHD before day 28 after HSCT, time-frame that in our cohort of patients always resulted free from infectious events, with PTX-3 plasma levels measured at the same time point (day 28) in patients who did not develop GvHD within day 28 (no GvHD group). This refined analysis confirmed our previous findings: the median PTX-3 level in 29 patients who developed GvHD within day 28 after HSCT was 35.80 ng/ml (range=11.21-847.44 ng/ml) and was significantly higher than the no GvHD group (PTX-3 plasma level=14.22, range=4.23-58.49 ng/ml, n=30). The difference between the two groups was statistically significant, Wilcoxon p-value<0.001.**Conclusions:** These preliminary results show that PTX-3 plasma levels increase very rapidly in patients experiencing acute GvHD, candidating this molecule as an easily measurable soluble factor useful to corroborate the clinical monitoring of the pathology. *Further studies* are needed to elucidate the role of PTX-3 in GvHD pathophysiology and to confirm its potential usage as GvHD biomarker in a larger cohort of patients.

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ADULT MOUSE HAEMATOPOIETIC STEM CELLS DO NOT GIVE RISE TO MESENCHYMAL STEM CELLS OR TUMOR ENDOTHELIAL CELLS

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Background. Hematopoietic Stem Cells (HSCs) are multi-potent cells that give rise to all hematopoietic cells over extended periods of time. It has been reported that HSCs could also generate non-hematopoietic cells, including mesenchymal cells and endothelial cells. This differentiation capacity could give rise to new and exciting therapeutic opportunities for HSC treatments. However, some research groups could not find the suggested differentiation capacity in their experiments and questioned the methodological exactness of the previously reported results. These incongruous findings led to an ongoing confusion and unabated debates about the differentiation potential of HSCs.**Aims**The aim of this study was, therefore, to investigate the potential of single HSCs to give rise to non-hematopoietic cells in vivo.**Methods**Highly purified HSCs isolated from mouse adult bone marrow (i.e. $EPCR^+$, $CD150^+$, $CD48^-$, $CD45^+$ (ESLAM) cells, 40% with long-term hematopoietic repopulating ability) were transfected overnight with a lenti-GFP virus and a single cell was then injected into each of 28 sub-lethally irradiated congenic *W41/W41* mice. Five mice, that showed a robust contribution (>1%) to all WBC for at least 16 weeks, then received a subcutaneous injection of Lewis lung carcinoma cells. After 10 days the resulting tumor was harvested and the tumor endothelial cells (TEC = $CD45^-Lin^-CD31^+$) were analyzed for GFP expression with fluorescence microscopy and fluorescence activated cell sorting (FACS). GFP^+ cells were further analyzed for viral integration sites and for X/Y chromosomes in mice where the ESLAM donor-host combination was sex-mismatched. Concurrently, bone marrow was harvested from the sacrificed mice and cultured using a protocol to selectively promote mesenchymal stem cell/fibroblast growth and differentiation. The MSCs were further differentiated into osteoblasts, adipocytes, and chondroblasts, which were then analysed for GFP expression with fluorescence microscopy.**Results**Between 2 and 32% of all TECs expressed GFP when analysed by FACS. Tumor sections analysed with fluorescence microscopy showed focal areas of GFP^+ endothelial cells that also expressed CD144. However, viral integration sites could not be detected on sorted GFP^+ TECs. Additionally, TECs from mice that received a sex mismatched cell, showed a triple-X pattern, which was also found in the Lewis lung carcinoma line. Bone marrow MSCs were entirely GFP^-, and so were the MSC-derived adipocytes and osteoblasts. Chondroblasts could not be analyzed definitively. Interestingly, $CD45^-Lin^-CD31^-GFP^+$ cells could be found in the bone marrow of positively engrafted animals but their exact relationship to other hematopoietic cells and their biological function remains to be determined.**Summary/Conclusions**Evidence of tumor endothelial cells or bone marrow mesenchymal stem cells could not be obtained from rigorously analyzed recipients of single HSCs in spite of FACS and fluorescence microscopy evidence of HSC progeny in tumors at sites suggestive of endothelial contributions. On the other hand, our data clearly demonstrate an ability of HSCs to generate $CD45^-Lin^-CD31^-$ bone marrow cells of unknown biological function and significance.

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OUTPATIENT AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MALIGNANT HEMATOLOGIC DISORDERS

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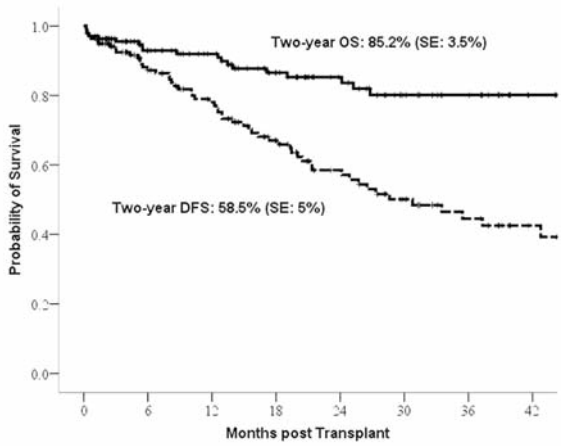
Background. High-dose chemotherapy with autologous stem cell support is utilized for the treatment of a variety of malignancies including Multiple myeloma, Hodgkin/non-Hodgkin's lymphoma and AML. The purpose of this study was to describe the time of engraftment, event-free and overall survival rates in outpatient cases treated with autologous stem cell transplantation in our center. **Methods.** From December 2005 to October 2011, medical records of 137 outpatients (97 MM, 24 HL, 10 NHL and 6 AML) were reviewed. Median age was 47 years (15-65). Complete remission was achieved in all patients and no significant evidence of organ failure was observed. They received conditioning regimen (melphalan for MM, CEAM for NHL and HL, busulfan and etoposide for AML) and stem cell infusion in the hospital. The first day after SCT, patients were discharged and then followed-up by outpatient SCT team. If the patients developed febrile neutropenia, rehospitalization was required. After sepsis workup and chest x-ray performance, they received the first dose of antibiotic in the hospital and then the therapy continued at home.**Results.** With a median follow-up of 20.5 months (1-68), the 2-year overall and disease-free survivals were 85.2% and 58.5%, respectively. Median time to ANC and PLT engraftments were 11.3 (rang: 8-26) and15 days (rang: 8-78), respectively. 118 patients are still alive while 4 patients due to transplant-related complications died during the first month following SCT. Relapses occurred in 48 patients. **Conclusions.** The results of the study show that autologous SCT in outpatient cases with malignant hematologic disorders is feasible. Further studies with longer follow-up are required to achieve reasonable results.



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OVER EXPRESSION OF PML CAN INHIBIT PROLIFERATION AND PROMOTE OSTEOGENIC DIFFERENTIATION IN HUMAN MESENCHYMAL STEM CELLS

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Background. With highly potential clinical application of human Mesenchymal Stem Cells (hMSCs), how to get more exogenous MSCs comes to be a new challenge. PML is an important regulator on many fundamental processes, such as apoptosis, cellular proliferation, senescence, cell cycle regulation. In our previous studies, we found PML is highly expressed in MSCs, but very low in tumor cells. We were interesting if PML has some special role on regulating proliferation and/or differentiation of MSCs. In this study, we explored the role of PML in MSCs.**Aims.** Our aim is to investigate the expression and localization of PML in MSCs and how PML regulates the proliferation and differentiation of MSCs. **Methods.** After approval by institutional review board, MSCs were isolated from the**bone** marrow of volunteers Ficoll-paque and its phenotype was confirmed by flow cytometry. Experiments were duplicated in 3 different and randomized samples. QRT-PCR and western blot was used to detect PML mRNA and protein expression. MTT assay and flow cytometry was used to analyse the proliferation of MSCs. PML expression plasmid plenti-6.3/PML and control vector (Invitrogen) were transfected into MSCs. MSCs were cultured with osteoinductive medium (Sigma-Aldrich) for two weeks and cells were collected on day 7 and day 14. Differentiated cells were identified with vonkossa staining and ALP activity analysis (KeyGEN). Ostoblast-specific genes were examined by QRT-PCR and Western blot after PML over expression in MSCs on day 7 and day 14. The location of PML protein during cell proliferation and differentiation was detected by immunofluorescence.**Results.** Isolated MSC cells were uniformly positive for CD29,CD44, CD166 and CD105, while negative for CD34, CD45 and HLA-DR. Compared to 293T cells, PML gene was highly expressed in MSCs, and increased time-dependently along with cell proliferation. The size of PML-NBs in MSCs was also increased during cell proliferation. After over expression of PML, the proliferation of MSCs was inhibited by more than 70% ($P<0.05$). However P27 was significantly lower and cyclin-D1 had an obvious increase in the plenti6.3-PML transfected cells compared with control. Also we found caspase-3, caspase-9 and PARP were not obviously increased with PML over expression. These indicate that over expression of PML can inhibit the proliferation of MSC cells, which is not mediated by cell cycle arrest or cell apoptosis. On day 14, MSCs of osteogenic differentiation were positive for vonkossa staining, and the expression of PML increased time-dependently. During cell osteogenic differentiation, PML-NBs were bigger but unequal in size compared with control. After over expressed with transfected PML, the relative mRNA expression of osteoblastic differentiation-related genes (SPP1□IBSP□BMP2) were up-regulated in MSCs, while the activity of ALP increased, which implied that the capacity of cell osteogenic differentiation was enhanced by PML.**Conclusion:**Our data suggests that PML regulate in MSCs as an inhibitor of cell proliferation but a promoter of cell differentiation. Our findings might give a new sight on better clinical application of MSCs..

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IMPACT OF REGULATORY T CELLS (TREG) ON EFFICACY OF AUTOLOGOUS AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: PRELIMINARY STUDY

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Background. CD4+CD25+Treg are largely responsible for suppressing GvH reactions in patients undergoing alloHSCT, thereby preventing the development of GvHD, facilitate engraftment and determine the effect of GvT. In the autologous setting and cancer evolution model Treg are supposed to actively participate in counteracting of antitumor immunity, contributing to the accelerated growth kinetics and tumor promotion. **Aims.** To assess reconstitution of hematopoiesis, CMV reactivation, aGvHD occurrence and overall survival in patients who underwent autoHSCT or alloHSCT according to the percentage of Treg during the peritransplant period. **Methods.** The study was conducted in 25 patients (M/F=13/12) aged from 20 to 59 years (median 37.7), including 9 with a diagnosis of AML, 1 - ALL, 7 - CML, 4 - HD, 2 - MM, 2 - NHL. AutoHSCT and AlloHSCT were performed in 11/25 and 14/25 patients, respectively. Treg cells CD4+CD25+ were determined by flow cytometry in all patients before transplantation and at +5, +10, +15 and +20 day post-transplant. *Results.* In patients undergoing autoHSCT neutrophil engraftment (ANC> $1.5x10^9$/L) was observed from 11 to 17 days post-transplant (median 12.8), platelet engraftment (PLT> $50x10^9$/L) - from 13 to 35 days (median 21,7). CMV reactivation was not confirmed in any patient of this group. The median percentage of Treg cells was as follows: before autoHSCT - 4.5%, in 5 days after the transplantation - 8.4%, at day +10 - 10.2%, at day +15 - 10.8%, at day +20 - 5,4%. The 5-year OS was 77%, median survival time (MST) was 57.6 months in median follow-up of 74.4 months. In 3/11 (33%) patients who had a relapse after autoHSCT (median TTP 3.6 months) Treg tended to increase in +5, +10 and +15 day (median 13%, 27.5 % and 15.5%), MST reached 18.3 months.In patients after alloHSCT neutrophil engraftment time extended from 14 to 26 days post-transplant (median 17.3) and platelet engraftment - from 19 to 39 days (median 28.5), respectively. The percentage of Treg was: before alloHSCT - 4.8%, at day +5 - 8.7%, at day +10 - 9.1%, at day +15 - 13.1%, at day +20 - 6%. 5-year OS was 7.1%, MST - 36 months in median follow-up of 67.5 months. In 4/14 (28.5%) patients who developed CMV reactivation, a higher values of Treg were observed, successively - 5%, 7%, 11.6%, 19%, 7.5%, MST - 45,7 months. aGvHD occurred in 6/14 (42.8%) patients, the Treg percentage presented levels - 4.6%, 9.8%, 10.8%, 12%, 5.3% and at day +15 and +20 reached values lower than reported in patients without aGvHD, while the MST was 27.6 months. Relapse of disease was observed in 5/14 (35.7%) - TTP = 23.4 months, MST 34 months - in this group analysis revealed a bias that Treg percentage were lower before alloSCT, at day +5 and +10, and higher at day +15, +20, comparing to the patients without relapse. *Summary.* Labelling of Treg cells percentage might be useful in the assessment of post-transplant course, especially in the risk of disease recurrence, but it requires further investigations in a larger group of patients.

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MANAGEMENT OF HEMANGIOMAS: A SINGLE CENTER EXPERIENCE

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Background. Hemangiomas are benign neoplasms of the vasculature characterized by abnormal growth of endothelial cells.**Aims.** To share our experience of 185 (F/M: 2.4) patients with hemangiomas followed between 2003-2011 at Cerrahpasa Medical Faculty, Pediatric Hematology Unit in Istanbul Turkey.**Results.** Of the 185 patients, 40% presented at birth. Onehundredtwenty eight (69%) had a single lesion whereas 31% had multiple lesions. In patients with a single lesion; 87 (68%) had a cutaneous hemangioma in the head and neck region (44 at eyelid and/or around eye). In 57 patients with multiple hemangiomas; 46 had a lesion in the head and neck region, 21 had a visceral lesion (4 liver, 1 spleen, 3 larnyx, 2 oropharynx, 4 parotid gland, 3 vertebrae, 2 internal jugular vein, 2 common carotid artery). Most of the patients (n:94) were followed by a "watch and wait"policy and 40 of them did not require any medical or surgical intervention as the lesion convoluted on follow-up. Only 6 (3%) patients required surgery to prevent the life-threatening complications and complications interfering normal physiology. Nine patients with lesion around eye required intralesional steroids to prevent visual disturbance. In 7 patients with an open, bleeding lesion local imiquimod was used with a successful result in 7-10 days. Bleomycin was used as a sclerosing treatment in 2 patients with a giant hemangioma in the tongue. In 21 patients with multiple diffuse lesions causing cosmetic problems, interferon was given. Medical treatment was given in other patients on follow-up due to growth, ulceration, bleeding or persistance of the lesions at older ages; 14 were treated by systemic steroids, 73 by propranolol and 39 by combination therapy due to insuffient response. Propranolol was the first choice of treatment in patients diagnosed after 2008 regarding the new literature. All patients treated by propranolol were evaluated by echocardiography and electrocardiograpy before and during therapy, no cardiac side effects were noted. One patient under treatment presented with increased sweating and was found to have hypoglycemic attacks associated with propranolol during periods of restricted oral intake. The drug was restarted increasing the frequency of breastfeeding with no further hypoglycemia attacks.**Conclusion:** The excellent clinical outcome and apparent lack of side effects of propranolol makes it a good choice as a first-line treatment for hemangiomas especially in young patients who may have interferance in vaccination with steroid treatment. We would like to call the attention that hypoglycemia may be noted in infants under propranolol in restricted periods of feeding.

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CLINICAL FACTORS AND THROMBOPHILIA AS RETHROMBOSIS RISK FACTORS

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Background. predictive factors of rethrombosis can help to stratify patients with venous thromboembolism and to apply for the best available treatment. **Aims.** to determine the relationship between rethrombosis and various clinical and thrombophilic factors. **Methods.** this is a nested case-control retrospective cohort study including 242 patients from the spanishprovince of Soria, with a history of venous thrombosis recruited between January 1998 and December 2010. Inclusion criteria: diagnosis of venous thromboembolism by objective methods three months before entering the study; spontaneous thrombosis (defined as that ocurred in patients not exposed in the 60 days prior to trauma, immobilization, surgery, pregnancy / puerperium or oral contraceptive use); less than 80 years of age; no previous history of chronic liver disease or kidney failure; no active neoplasia at the time of thrombosis; no oral anticoagulation within 30 days before entering the study.Determinations by STA-R coagulometer: antithrombin, protein C levels and clotting protein S, free protein S, resistance to activated protein C, factor VIII levels and lupus anticoagulant. Mutations in factor V Leiden, and prothrombin gene were determined by PCR. Levels of fasting plasma homocysteine were also tested. Positive samples, except the mutant ones, were confirmed by a new determination at 3 months. **Results.** 212 patients were included: 119 (56.1%) males and 93 (43.9%) female. Median age at diagnosis of the first event was 41 years (9-79). Median number of episodes was 1. First thrombotic event: 112 (52.8%) deep vein thrombosis (DVT), 59 (27.8%) proximal deep vein thrombosis, 26 (12.3%) pulmonary embolism, 10 (4.7%) cerebral venous thrombosis, 4 (1.9%) mesenteric venous thrombosis and 1 (0.5%) renal vein thrombosis. 119 (56.1%) were spontaneous thrombosis and 93 (43.9%) secondary. Re-thrombosis risk was 2.7 times higher in the group aged ≥ 55 years. Patients who developed DVT, proximal or distal had high risk of recurrence, with an OR of 5.8 (1.7 to 19.7, p = 0.002). The presence of a genetic or acquired thrombophilic factor was associated with an increased risk of rethrombosis with an OR of 2.5 (1.3 to 4.8, P = 0.004). A higher risk of re-thrombosis was observed in patients with positive lupus anticoagulant OR of 5.3 (1.3 to 22.2-, p = 0.01), elevated homocysteine levels (> 12 mcmol / L) OR 3.7 (1.1 to 12.4-, p = 0.02) and high Factor VIII levels (> 150 IU / dL) OR 2.6 (1.2 to 5. 3, p = 0.008). **Summary and Conclusions.** our study shows a statistically significant relationship between certain thrombophilic disorders (factor VIII, presence of lupus anticoagulant, elevated homocysteine levels) and risk of rethrombosis. The age and type of initial thrombosis was also statistically significant correlated with the risk of thrombosis recurrence.

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FREQUENCY, CLINICAL PATTERN AND OUTCOME OF THROMBOSIS IN CANCER PATIENTS

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Background. Thrombotic risk is increased in patients with cancer and there are important implications for cancer patients who develop VTE.**Aims.** We undertook this study to determine the frequency, clinical patterns, and outcome of thrombosis in Saudi patients with cancer. **Methods.** Cancer (solid tumors and lymphoma) patients who developed thrombosis from January 2004 to December 2008 were studied retrospectively. Demographics and characteristics related to thrombosis and cancer along with the outcome were evaluated.**Results.** A total of 701 patients with cancer were seen during the study period. VTE was diagnosed in 47 (6.7%) patients (median age 52, range 18-80 years). Lower limbs DVT was the most common type of thrombosis seen in 47% patients, followed by PE in 19% patients and 19% patients had both DVT & PE. Thrombosis was symptomatic in 70% patients while it was an incidental finding on routine workup in 30% patients. 38% patients were diagnosed to have cancer and VTE at the same time, and 47% developed VTE during the course of disease after the cancer diagnosis. Majority of the VTE post cancer diagnosis occurred during the first year (median 4 months, range 1-14 months). Additional risk factors for VTE were present in 22 patients and 14 of these patients were receiving chemotherapy at the time of thrombosis. Only 5 (10.6%) patients were receiving thrombo-prophylaxis at the time of VTE diagnosis. Most common type of cancers associated with thrombosis were breast cancer, non-Hodgkin's lymphoma and lung cancer. Majority of the patients (33, 70%) with VTE diagnosis had advanced stage of cancer. After a median follow-up of 13 (range 0.5-60) months, 37 (79%) patients have died. Almost all the patients with stage 3 or 4 cancer and thrombosis died except for 2 patients with non-Hodgkin's lymphoma.**Conclusions:** Thrombotic complications can develop in a significant number of Saudi patients with cancer and almost half of the patients have additional risk factors for VTE. Thrombosis is usually associated with advanced disease and can be asymptomatic in almost one-third of patients. Thrombo-prophylaxis in cancer patients is under-utilized. Community based studies are needed to accurately define the extent of this problem and to develop effective prophylactic strategies.

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RECURRENT CEREBRAL AND ABDOMINAL THROMBOSES AND PULMONARY EMBOLISM (PE) IN A NON-TRANFUSION DEPENDENT PATIENT WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA. EFFECTIVENESS OF ECULIZUMAB TREATMENT

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Background. Paroxysmal nocturnal hemoglobulinuria (PNH) is a rare, genetic, hematopoietic stem cell disorder characterized by chronic uncontrolled terminal complement activation, causing hemolysis, platelet activation, and inflammation, and ultimately leading to serious morbidities. Thromboembolism (TE) is among the most dangerous complications of PNH. **Aims.** To present a case highlighting severe recurrent TE occurring in a non-transfusion dependent PNH patient, despite prophylactic warfarin anticoagulation. *Results.* In July 2004, a 36-year-old woman in her second pregnancy presented with isolated low platelet count ($37x10^9$/L). Routine diagnostic work-up was unremarkable includ-

ing tests recommended to detect causes of secondary thrombocytopenia. LDH level was between 1 and 1.5 ULN, a potential clue to an early diagnostic workup to exclude PNH Bone marrow aspiration was normal and idiopathic thrombocytopenic purpura was diagnosed. Despite treatment with high-dose steroids, intravenous immunoglobulin and cyclosporin A her platelet count did not improve. At term, a Caesarean section was performed along with a splenectomy. Following a massive post-partum hemorrhage requiring packed RBC transfusions the patient was discharged in September 2004 with Hb 11.6 g/dL, but still low platelets ($35x10^9$/L). Two years later the patient was hospitalized in the department of neurology of another hospital after experiencing drowsiness, headache and transient aphasia. Cerebral CT scan revealed thrombosis of the sagittal sinus, moreover hepatic enlargement, ascites, due to Budd Chiari syndrome ,along with asymptomatic PE were detected and warfarin treatment initiated. The patient was diagnosed with thrombophilia of unknown origin. Platelet count ranged between 50 and $100x10^9$/L. During follow-up, a series of diagnostic tests, including bone marrow biopsy (cellularity 15%), tumor markers and markers of congenital and acquired thrombophilia were performed. PNH was diagnosed by flow cytometry (99% GPI-linked CD14 and CD55 defect). The patient developed hepatic vein thrombosis in 2007 and was hospitalized again in January 2009 with persistent abdominal pain while on warfarin and with INR within the therapeutic range. D-dimer level was 5290 ng/ml and LDH 2N , Abdominal Doppler US revealed massive portal vein thrombosis with cavernoma of the liver. Eculizumab therapy was started in June 2009, as recommended in patients with recurrent abdominal and cerebral thromboses (Brodsky, Blood 2011), and because oral warfarin had proven ineffective. The subsequent clinical course was unremarkable with no further reports of abdominal pain, or TE and normalization of all hematological values (LDH: 159-364 IU/L; Hb: 12.6 g/dL; platelets: 231,000/mm^3). D-dimer level range between N and 2N.*Conclusions*: We suggest that in patients who have a history of potentially devastating recurrent venous thrombosis eculizumab treatment must be considered, even when haemolysis is not serious enough to require blood transfusion. In this particular patient, who had experienced at least 3 major thrombotic events over a 5 year period, there has been no recurrence since eculizumab was started over 2 years ago. (Medical writing support was provided by Åsa Lommelé.)

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THROMBOPHILIA IN CHRONIC HEMOLYTIC ANEMIA PATIENTS: ANALYSIS OF GENETIC PREDISPOSING FACTORS

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Background. Genetic thrombophilia as a contributing factor for hypercoagulability among patients suffering from hemogloinopathies has been Recently studied. Thromboembolic events as transient ischemic cerebral attacks, strokes, pulmonary embolism, deep venous thrombosis, and portal vein thrombosis, have been observed in thalassmia patients.The hypercoagulable state has been attributed to a wide variety of hemostatic alterations including platelet hyperaggregability, protein C and antithrombin deficiency and procoagulant alterations of red cells. Few studies have evaluated the impact of prothrombotic polymorphisms in thalassemia patients. **Aims.** to determine the frequency of selected prothrombotic gene polymorphism (FV, FII, MTHFR) in a cohort of beta-thalassemia and sickle cell disease patients and to assess their impact on the precoagulant state, clinical and laboratory parameters. **Methods.** Study included 80 patients with chronic hemolytic anemia divided into:Group A: Thalassemia major patients (n=30) with age ranging from 11- 33 years with a median age of 20 years.Group B: Thalassemia intermedia patients (n=20) with age ranging from 5 - 18 years with a median age of 10 years.Group C: Sickle cell disease patients (n=30) with age ranging from 8 - 19years with a median age of 9 years. One-hundreds healthy age and sex matched subjects were served as controls.*Patients were subjected to history taking, laying stressing on:*demographic data,consanguinity,history of splenectomy.Symptoms suggestive of previous attack of thromboemolic manifestation,family history of thrombotic disorders,blood transfusion details and history of vasooclusive crises. L*aboratory Investigations included* :Complete blood count,PT, PTT and mean serum ferritin level.Polymerase chain reaction analysis of a sample of DNA for factor V Leiden G1691 A, factor II G20210A, and MTHFR C677T .Results. heterozygous Factor V leiden mutations were present in 28.7% of the studied patients with the highest frequency in thalassemia intermedia compared to thalassemia major and SCD patients(16%,9%,3.7%,p=0.02). Heterozygous Factor II 20210 mutation was present in 4.8%, all of them in SCD patients. Heterozygous MTHFR C677T mutation in 33.3% with the highest frequency in thalassemia intermedia patients compared to thalassemia major and SCD (20%,11%,2,3% respectively,p=0.01). MTHFR C677T homozygous mutation were found in 4.8% and all were thalassemia major patients. While factor V lieden, MTHFR mutations were present together in 9.5% of cases (7% in thalassemia intermedia and 2.5% were SCD patients).Patients with β-thalassemia major and SCD with positive mutations had higher higher incidence of past history of thrombotic events (p=0.02).Cases with β-thalassemia major showed significantly higher rate of heterozygous (G/A) when compared to SCD and TI(p=0.04).Patients with β-thalassemia intermedia showed significantly higher rate of inherited thrombophilia mutation than those with thalassemia major (P=0.01) with an odds ratio of 2.7 (95%CI 0.84-8.5). TM patients with positive mutations showed significant higher level of mean total leucocytic count and short values of PT when compared to cases with no mutation(P<0.05). All TI patients with positive mutations had past history of venous thrombosis. SCD patients with positive thrombophilic mutations had higher frequency of vasco-occlusive crisis(p<0.05). Conclusions:thrombophilia work up is necessary in patients with chronic haemolytic anemia especially thalassemia and sickle thalassemia patients presenting with past history of thromboembolic events.Thrombophilic mutations is related to vasoocclusive crisis risk in SCD patients .

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CANCER PATIENTS REQUIRING INTERRUPTION OF LONG-TERM WARFARIN BECAUSE OF SURGERY OR CHEMOTHERAPY INDUCED THROMBOCYTOPENIA: THE USE OF FIXED SUB-THERAPEUTIC DOSES OF LOW-MOLECULAR WEIGHT HEPARIN

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No data is present regarding the management of cancer patients requiring interruption of long-term vitamin-K antagonist (VKA) therapy. For this purpose, we tested the efficacy and safety of fixed doses of low-molecular weight heparin (LMWH) in substitution of VKA because of invasive procedures or chemotherapy-induced thrombocytopenia. In cancer patients on VKA, therapy was discontinued 5 + 1 days before surgery or chemotherapy. Heparin was given at prophylactic dosage in patients at low risk and at fixed sub-therapeutic doses (3,800 or 4,000 UI anti-FXa, b.i.d.) in those at high-risk for thrombosis. LMWH was reinitiated 12 hours after surgery and VKA the day after. In patients receiving chemotherapy, LMWH was reinitiated 12/24 h after obtaining a stable platelet count > 30,000 mmc^3, and VKA after a stable platelet count > 50,000 mmc^3.

Table 1. Baseline characteristics.

Patients characteristics (n=156)	
Mean age (range, y)	66.6 (32/89)
M/F (%)	84/72
Weight, mean ± SD (Kg)	75.4 ± 16.5
Solid cancer, n (%)	98 (62.8)
Haematological cancer, n (%)	58 (37.2)
Advanced/metastatic cancer, n (%)	101 (64.7)
Bridging therapy with nadroparin, n (%)	71 (45.5)
Bridging therapy with enoxaparin, n (%)	79 (50.6)
Bridging therapy with others heparin compounds, n (%)	6 (3.8)
Low-risk for TE	88 (56.4)
- Patients on chemotherapy-induced thrombocytopenia	22*
High-risk for TE	68 (43.5)
- Patients on chemotherapy-induced thrombocytopenia	17**
Venous thromboembolism, n (%)	52 (33.3)
- Events lasting < 3 months, n (%)	28
- Events lasting > 3 months, n (%)	24
Atrial fibrillation without previous stroke (AF-NoAT), n (%)	48 (30.7)
Atrial fibrillation with previous stroke (AF-AT), n (%)	21 (13.4)
Prosthetic aortic/mitral valves (PAV), n (%)	19 (12.1)
Others (arterial hypertension, dilatative myocardiopathy, valvulopathy, myocardial infarction, coronary artery by-pass graft), n (%)	16 (10.2)

*16 (72.7%) had haematological malignancies

**11 (64.7%) had haematological malignancies

Thromboembolism and major bleeding events were recorded from the time of VKA suspension to 30 + 2 days post-procedure, or until the next chemotherapy. Overall,156 patients (56.4% at low risk and 43.5% at high risk for thrombosis) were enrolled; 34.6% underwent major surgery, 40.4% non-major surgery, and 25% chemotherapy. Thrombotic events occurred in 5 patients (3.2%, 95% CI 1.41-7.27), 4 belonging to the high-risk and 1 to the low-risk group. Major bleeding occurred in 5 patients (3.2%, 95 CI 1.41-7.27), all belonging to the high-risk group (3 during major surgery and 2 during chemotherapy). In conclusion, LMWH given at fixed sub-therapeutic is a feasible and relatively safe approach for bridging therapy in cancer patients on long-term VKA.

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EFFECTS OF DIAGNOSTIC ULTRASOUND-MEDIATED MICROBUBBLE CONTRAST AND UROKINASE ON AUGMENTATION OF THROMBOLYSIS AND OPTIMIZATION OF THE MAJOR PARAMETERS: AN IN VITRO STUDY

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Objective To investigate the efficacy of diagnostic ultrasound and microbubble contrast (MB) on enhancing thrombolysis combined with urokinase(UK) and explore the major parameters to confirm the optimal combination for thrombolysis *in vitro*. **Methods.** Four types of standardized red thrombus were prepared *in vitro* for the present study, including 3-hour-old (3h), 6-hour-old(6h), 12-hour-old(12h), 24-hour-old(24h), respectively. The major parameters for the designed experiments included transmit powers of ultrasound (factor A, 5%, 25%, 50%, 100%), MB volumes (factor B, 50μL, 100μL, 200μL, 400μL), UK concentrations (factor C, 100U/ml, 200U/ml, 400U/ml, 800U/ml), and lysis time (factor D,10min, 20min, 30min, 40min), respectively. Then an orthogonal array experimental design (OAD) based on four parameters above and four levels[$L_{16}(4^5)$]was employed to optimize the thrombolysis conditions in our study. The blood clots were weighed before(W_0) and after(W_1) experiments to determine lysis efficiency(LE) based on the following formula, LE=[(W_0 − W_1)/W_0]×100%. Sixteen thrombolysis experiments were performed based on the $L_{16}(4^5)$matrix, and for each level, three experiments were repeated per protocols. During the procedure of thrombolysis, the diagnostic ultrasound frequency was fixed at 1.82MHz. The histopathological changes measured by HE staining and scanning electron microscope (SEM) were carried out to observe the clots before and after thrombolysis. The direct observation analysis was used to optimize and the analysis of variance (ANOVA) was used to assess the results according to the $L_{16}(4^5)$ matrix. **Results** The HE staining and SEM observation of thrombolysis under the following experimental condition of 5% ultrasound transmit power, 400μL MB volume, 800U/ml UK concentration, 40min lysis time showed remarkable disaggregation of fibrin nets. Four factors above were shown to be statistically significant in each type of thrombus at different levels (all conditions, P<0.01). Among them, UK concentrations (factor C) was one of the most significant parameters for four types of clots. The optimal scheme was determined as the C_4-D_4-A_1-B_4 mode, UK concentration 800U/ml, lysis time 40min, transmit power 5%, MB volume 400μL, respectively. The LE curves of each factor for 3h clots were superior to the other LE curves for 6h, 12h and 24h clots in turn. **Conclusions** 1.82MHz diagnostic ultrasound and microbubble contrast may be applied to augment thrombolysis *in vitro* even transmit power was lowest to 5%. Under the condition of fixed ultrasound frequency, the LE of thrombus increased with increasing UK concentrations, lysis time and MB volumes, while decreased with increasing thrombus ages. Therefore, the older aged thrombi may not obtain better thrombolytic rates.

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MEAN PLATELET VOLUME AND PLATELET COUNT: OVERSEEN MARKERS OF HIGH ON-TREATMENT PLATELET REACTIVITY AND WORSE OUTCOME IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background. Mean platelet volume (MPV) is a marker of platelet activation and turnover. Platelet count (PC) and platelet hematocrit (PCT) correlate with total volume of platelet cytoplasm rich of proaggregatory substances. It can be expected that increase in these values leads to worse response to antiplatelet treatment and worse outcomes.Objectives: The primary aim of the study was to assess the relationship between MPV, PC, PCT and responsiveness to the antiplatelet treatment. The secondary aim was to assess the relationship between MPV, PC, PCT and mortality in patients with acute coronary syndrome.Patients/methods: We performed a cohort study of 190 patients with acute coronary syndrome. In these patients the MPV, PC and PCT were measured. The response to aspirin and clopidogrel was simultaneously assessed by the impedance aggregometry-based assay.Results. Patients with high MPV were in higher risk of dual poor responsiveness (HR 2.53, 95 % CI 1.15-5.37, p<0.05) and poor responsiveness to aspirin (HR 2.38, 95 % CI 1.37-3.92, p<0.01). Patients with high PC were in higher risk of poor responsiveness to clopidogrel (HR 2.60, 95 % CI 1.07-4.33, p<0.05). The three-year mortality was increased in patients with high MPV (25.7% vs. 13.5%, p<0.05). **Conclusions.** MPV and PC are useful tools in prediction of response to antiplatelet treatment. Increased MPV is a predictor of increased long-term mortality in patients with acute coronary syndrome.

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SOME HEMOSTATIC BIOMARKERS IN PATIENTS UNDERGOING LASER INTERVENTION OF LEG VARICES

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Background. The thermal effect of endovenous laser ablation (EVLA) induces endothelium damage and intraluminal thrombus formation in leg varicose veins. In the case of thrombus extension from superficial to the deep venous system this can bear a risk of pulmonary embolism.**Aims.** The study was aimed to verify the changes of molecular hemostatic biomarkers in patients undergoing laser intervention of leg varices. **Methods.** Twenty patients (age 43,8 ± 11,6 years; 14 female, 6 male) undergoing EVLA due to incompetence of great saphenous vein were enrolled in the study. All patients gave their informed consent with participation in the study. Plasma levels of D-dimers (DD), prothrombin fragments 1+2 (F1+2), soluble P-selectin (sP-selectin), soluble thrombomodulin (sTM), von Willebrand factor (vWF), factor VIII (FVIII) and plasminogen activator inhibitor (PAI-1) were measured from the venous blood before EVLA as well as on 1st day, 9th day and 1 month after EVLA.**Results.** DD plasma levels were significantly increased on the 9th day after EVLA compared to the DD levels before EVLA and on the 1st day after EVLA (1,36±1,36 vs. 0,25±0,14 mg/l; p<0,05 and 1,36±1,36 vs. 0,61±0,44 mg/l; p<0,05, respectively). Moreover, the DD plasma levels taken 1 month after EVLA were significantly decreased in comparison with DD levels on the 9th day after EVLA (0,48±0,29 vs. 1,36±1,36; p<0,05). F1+2 plasma levels increased on the 1st and 9th day after EVLA but without significance. vWF plasma levels on the 1st day after EVLA were significantly increased compared to the vWF levels 1 month after EVLA (1,17±0,25 vs. 0,99±0,21 U/ml; p<0,05). FVIII plasma levels on the 9th day after EVLA was also significantly increased in comparison to the FVIII levels before EVLA (1,10±0,33 vs. 0,92±0,20 U/ml; p<0,05). The sP-selectin, sTM and PAI-1 plasma levels did not change significantly. **Summary and Conclusions.** Our preliminary results suppose that EVLA did not lead to the platelet activation in systemic circulation and the endothelial dysfunction after EVLA seems to be expressed in systemic blood only as an endothelial activation (increased vWF but not sTM). The limited increase of DD levels can reflect that surgical trauma caused by EVLA is modest.*Acknowledgement: This study was supported by the grant Vega 1/0016/12, project CEVYPET (ITMS 2622012053) and project CEPV II (ITMS 26220120036).*

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LONG-LASTING OBSERVATION OF A GROUP OF PATIENTS WITH RARE (HOMOZYGOUS OR COMBINED) TYPE OF THROMBOPHILIA

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Summary. The aim of the study was a long-lasting observation of a group of patients with rare (homozygous or combined) thrombophilias, selected from a group of 350 patients with all types of thrombophilia.The study group consisted of 29 patients (18 women and 11 men) of the mean age of 38 years (22 - 71 years), who experienced at least one venous thromboembolic (VTE) episode in the past and were diagnosed to have a rare type of thrombophilia (8,3% of all thrombophilias).20 patients were on long-term secondary antithrombotic prophylaxis with vitamin K antagonists (INR 2,0 - 3,0); 5 patients were treated with enoxaparin for one year after the acute VTE episode, and with vitamin K antagonists afterwards; 4 patients were without secondary prophylaxis. Patients treated with oral anticoagulants received enoxaparin (50% of therapeutic dose)

for a week before blood drawing.Type of thrombophilia: 8 patients were carrying the homozygous factor V Leiden mutation (*G1691A*, FVL), 6 patients - the heterozygous factor V Leiden mutation along with heterozygous prothrombin gene mutation (*G20210A*), 7 patients had hereditary thrombophilia and coexisting antiphospholipid antibodies, 6 patients other combined thrombophilias (4 patients - Factor V Leiden or prothrombin gene mutation, along with protein C deficiency or increased activity (>150%) of factor VIII; 2 patients - protein S deficiency along with an increased activity of factor VIII).7 out of 8 patients (87,5%) with homozygous FVL mutation experienced their first thrombotic event before the age of 40. Family history of VTE has been noticed in 35% of patients with hereditary thrombophilia.During the observation time, which lasted for 1 to 14 years (mean 5 years) in 7 patients (24%) the recurrence of VTE has been diagnosed, in three of them during secondary prophylaxis. We observed atypical localization of thrombosis in five patients (17%), in two of them it was coexisting with proximal deep vein thrombosis of lower extremities. None of the patients had splanchnic venous thrombosis. Our most severe case was a 49 - year old patient with homozygous FVL mutation and coexisting antiphospholipid antibodies. She experienced three spontaneous cerebral ischemic strokes (first at the age of 36 years) and thrombosis of common jugular vein, left axillary vein and left subclavian vein after hysterectomy. **Conclusion**s. Homozygous or combined thrombophilias, diagnosed in about 8% of patients with all types of thrombophilias, are characterized by an early onset of VTE, and in a long-lasting observation by a tendency to recurrence, in some of patients even during secondary antithrombotic prophylaxis, as well as by atypical localization of thrombosis.**Keywords**:thrombophilia, venous thromboembolism, recurrent venous thrombosis

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HEMOSTATIC PARAMETERS AND LIPID PROFILE IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM: ASSOCIATIONS WITH PARATHYROID HORMONE

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Background. Primary hyperparathyroidism (P-HPT) represents a generalized disorder of calcium, phosphate and bone metabolism characterized by the increased secretion of parathyroid hormone (PTH) resulting in hypophosphatemia and hypercalcemia which represents the most common cause of hypercalcemia in the outpatient setting, in patients without an underlying malignancy. The diagnosis is frequently made in patients who are asymptomatic and present hypercalcemia and elevated levels of PTH. Patients with P-HPT present an increased risk for hypertension and cardiovascular disease. The influence of P-HPT and PTH in hemostasis and platelet function is not fully elucidated.**Aims.** The aim of the present study was to assess hemostatic parameters in patients with P-HPT in comparison to age-, gender and body mass index (BMI)-matched healthy controls and to explore the associations between these parameters with serum PTH, calcium and serum lipid profile. **Methods.** In a cross-sectional study between 2007 and 2011, we have evaluated eighteen patients with P-HPT due to a parathyroid adenoma, prior to any therapeutic intervention (parathyroid surgery) (15 women and 3 men) with a mean age: 49.05 ± 8.1 years (range: 33-64 years) and a double number of healthy subjects (30 women and 6 men) with a mean age: 51.02 ± 9.3 years (range: 32-66 years). Healthy subjects were matched (2 controls to one patient) on gender, age (±5 years), body mass index (± 1.5 kg/m^2) and year/month of diagnosis (±1 month). None of the subjects (patients and controls) presented any infectious and neoplastic conditions, diabetes mellitus, hypertension and dyslipidemia. Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, vWF, antithrombin III (ATIII), plasminogen, protein C and S, and D-Dimer were evaluated using immunonephelometry (Dade Behring). Lipid variables such as triglycerides, total cholesterol (C), LDL-C and HDL-C were also assessed. To evaluate thrombopoiesis, we have determined platelet indices (mean platelet volume, MPV; platelet distribution width, PDW; platelet count) using the Sysmex 9000 analyzer. PTH was determined using an electro-chemiluminescence immunoassay intended for use on Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, USA). Statistical analysis of data was performed with IBM-SPSS® statistical package version 20 for Windowssoftware.Results. Cases with P-HPT presented significantly higher mean fibrinogen levels (p=0.032), D-Dimer (p=0.042), MPV (<0.001), PDW (<0.001) and platelet count (p=0.002) than healthy controls. Moreover, cases exhibited statistically significant decreased levels of HDL-C (p=0.03) and significant increased levels of triglycerides (p=0.028), total cholesterol (p=0.047) and LDL-C levels (p=0.039). Moreover, PTH levels were positively associated with MPV (Spearman r=0.58, p=0.013) and D-dimer (Spearman r=0.41; p=0.024) in patients with P-HPT. We did not find any significant association between PTH and serum calcium with the other studied hemostatic and metabolic parameters. **Conclusions.** In conclusion, we have found that subjects with P-HPT present increased platelet parameters, platelet count, fibrinogen and D-Dimer which represent a potential hypercoagulable state. Elevated platelet activation and hypercalcemia in conjunction with coagulation and lipid perturbations may contribute to an increased risk of atherosclerotic complications observed in P-HPT. Serum PTH may account for the platelet morphologic changes observed in patients with P-HPT.

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CLINICO-DEMOGRAPHIC ANALYSIS AND OUTCOME OF CHILDHOOD THROMBOSIS IN PEDIATRIC HEMATOLOGY AND ONCOLOGY UNIT

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Background. Although thrombosis is rare in childhood, children with certain disorders may be at a higher risk.**Aims.** Our retrospective study was designed to evaluate patients with documented thrombotic events registered in Hematology -Oncology Unit over the last 3 years as regards clinical features, etiology, management and outcomes. **Methods.** The study included 30 patients (16 female and 14 male, age ranged from 1 month to 15 years; median 4.5 years) with clinical and radiological evidence of thrombosis. Data collection included clinical presentation, any identifiable risk factors for thrombosis including pre-existing hematological disorder as sickle cell anemia(SCA)/thalassemia, pre-identified cancer disorder and other risk factors including sepsis, immune disorders, catheter insertion, basic thrombophilia screening if done , chosen radiologic investigations, details of drug therapy and patients outcome.**Results.** Age at first thrombotic event was higher for occurrence of secondary thrombosis (median 6 years) than primary cases (median 8 months) (p=0.018). In 66.7% of patients, there was at least one identified risk for thrombosis, with cancer chemotherapy being the most frequent risk factors in 33.3% of patients(23.3% ALL-Pre B)then the presence of a pre-existing hematological disorder in 20% (10% sickle cell disease). In 33.3% of patients, inherited thrombophilic conditions were suggested (proven in 13.3% and undefined in 20%).Secondary thrombophilia presented with neurological symptoms (convulsions/decreased conscious level) related to central thrombosis in 70% of cases while inherited thrombophilias presented by gangrenous lesions (in 80%), especially small vessel disease presenting as purpura fulminans (P=0.001). No difference in the management between primary and secondary cases (56% of primary cases and 58% of secondary cases received a combination of low-molecular-weight heparin (LMWH) and oral anticoagulation) (p=0.063). A fixed dose was adopted for LMWH: 150 IU/kg/day for neonates and 100 IU/kg/day for older children. Fresh frozen plasma (FFP) was used mostly in primary thrombophilias (30%), and once in a patient with purpura fulminans secondary to sepsis. Two SCA patients with thrombosis were managed by exchange transfusion. Two studied patients with inherited thrombophilia were initially presented by neonatal stroke, their management included FFP followed by oral anticoagulation, and both experienced recurrence despite oral anticoagulant therapy. Recurrence occurred in 80% of patients despite oral anticoagulation. However, the recurrence was higher with primary thrombophilias (30%) compared to secondary cases(10%); yet no significant difference was found regarding residual effects with neurological deficit being the most common residual effect in both primary (20%) and secondary (31.6%) thrombophilias(p=0.139). Outcome of patients included normal (37%), neurological deficits (29.6%), recurrence (16.6%), amputation (7.4%) and death (16.6%). Out of the 16 patients who experienced central thrombosis, neurological sequelae included residual hemiparesis (33%), pschycosocial problems (33%), recurrent seizures (22%) and recurrent headache(11%) **Conclusion:** Compared to inherited thrombophilias; thrombosis secondary to an acquired risk factor tend to occur at an older age, commonly presented by central thrombosis with no significant difference between primary and secondary thrombosis as regards residual effects and recurrence rate. Further studies are warranted to determine proper duration of anticoagulant therapy to prevent possible recurrence.

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STUDY OF PLASMA FIBRINOGEN IN PREGNANT WOMEN WITH SEVERE PREECLAMPSIA

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Background. Preeclampsia is a complication of pregnancy constituting a major cause of maternal and fetal morbidity and mortality. Pregnancy is a hypercoagulable state with changes in procoagulant, anticoagulant, and fibrinolytic systems.In preeclampsia, there is a shift in the haemostatic balance towards a pro-thrombotic state, together with changes in endothelial function. It is a state of enhanced coagulation as evidenced by an increased amount of clotting factors in maternal circulation. **Aims.** To study the changes in plasma fibrinogen, in pregnant women suffering from severe preeclampsia, and compared with healthy normotensive pregnant women. To correlate its level with the severity of preeclampsia **Methods.** This study conducted at Alyarmouk teaching hospital / obstetric and gynecology department in cooperation with the national center of hematology.before start of the study a written informed consent was obtained from all patients and healthy controls.It included thirty five pregnant women in the third trimester of pregnancy with severe preeclampsia .Their age range between 18-41 years. A total of thirty five healthy pregnant women who were not in labour, their age and gestational age matched with the patients and normotensive throughout gestation were included as a control group.The patients who had any confounding conditions that could altered coagulation tests e.g placental abruption or previa, sepsis, stillborn or heavy vaginal bleeding, history of diabetes, renal disease, chronic hypertension, cardiovascular diseases, symptomatic infectious diseases , and use of anticoagulant drugs were excluded from the study. Blood samples were taken from both groups for measurement of Fibrinogen [by parallel line bioassay of coagulation factors].The student T-test and correlation coefficient study were used for statistical tests. **Results.** There was significant difference in mean plasma fibrinogen between patients with severe preeclampsia (5.10±0.83) and control group (4.24±0.61). (P value < 0.0001). characteristics of patients and healthy controls groups with statistical significance shown in Table 3. **Conclusions.** Plasma fibrinogen was significantly increased in patients with severe preeclampsia than control group and show significant direct linear correlation with the severity of preeclampsia.

Table 3: showing mean, standard deviation, range and P value of different parameters in pregnant women with severe preeclampsia and control subjects.

	Preeclampsia		Control		P value
	Mean±SD	Range	Mean±SD	Range	<
Age (years)	29.40±6.60	18-41	30.80±6.54	19-41	0.376
Gestational age (weeks)	35.97±2.79	30-40	35.74±2.90	30-40	0.738
Systolic blood pressure (mmHg)	174.42±15.84	160-210	112.00±9.0	100-130	0.0001*
Diastolic blood pressure (mmHg)	114.57±5.19	110-125	66.71±7.56	60-80	0.0001*
Mean arterial pressure (mmHg)	134.80±6.65	127-150	81.80±6.12	73-93	0.0001*
Fibrinogen (g/l)	5.10±0.83	3.5-6.5	4.24±0.61	3.3-5.3	0.0001*

*:Statistically significant difference ($p < 0.05$) from the controls

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ALGORITHM FOR ASSESSING THROMBOTIC RISK IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. If assessing the presence of thrombophilia markers is not a common determination, the risk of thrombotic events can be estimated by evaluating the history, clinical examination, blood count (including platelet count and mean platelet volume), blood lipids and the presence of metabolic syndrome components. **Aims.** We aimed to develop an algorithm to estimate the risk of thrombotic accidents in patients with CLL. **Methods.** We studied all 64 patients with CLL from their first hospitalisation in 2011, who were in the electronic filing system of the Hematology Department of Sibiu. We noted: gender, age, disease stage, platelet count, mean platelet volume, serum cholesterol, the possible presence of the metabolic syndrome components and a history of thrombotic events. We analyzed the correlations between the data obtained and the risk of thrombosis. **Results.** The mean age of the group was 66.93+/-10.70 years. Distribution by gender: women - 37 (57.81%), male - 27 (42.19%). The average number of platelets in peripheral blood was 192,837.21+/-83,277.20/mm^3. There was a moderate direct correlation between platelet count and thrombotic stroke in the history of patients (r=0.319). Those who have had thrombotic events have higher risk for new ones. There is a small but inverse correlation between the number of history of thrombotic events and the stage of CLL (r=-0.230). There was no correlation between thrombotic history and other analyzed data. It follows that the main thrombotic risk factor in our group is the increased number of platelets. Platelets are slightly, directly correlated with the number of components of metabolic syndrome (r=0.237). So, in early stages of disease, the presence of metabolic syndrome components could be an additional thrombotic risk factor. But, while the infiltration of bone marrow by leukemic cells grows, platelet count decreases, as reflected by moderate inverse correlation between platelet count and stage of disease (r=-0.343), and cholesterol decreases (average cholesterol 195.65+/-49.72mg/dl). There is a slight, direct correlation between platelet count and cholesterol (r=0.260). Disease stage correlates, also moderate and inversely, with cholesterol (r=-0.321), which in turn, correlates inversely, moderately, with the mean platelet volume, which was 10.59+/-0.84fl (r=-0.360). As a result, patients with advanced disease have a tendency to decrease in cholesterol (cholesterol is captured by leukemic cells and used for their proliferation), which correlates with increased mean platelet volume - a thrombotic risk factor. Between cholesterol and triglyceride there is a moderate direct correlation (r=0.407) and serum triglyceride correlates slightly and inversely with mean platelet volume (r=-0.140). Age, cholesterol and triglyceride correlates directly, slightly, with the number of components of the metabolic syndrome. In advanced disease, in addition to increased mean platelet volume, chemotherapy may contribute to increased thrombotic risk. **Conclusions**. In the early stages of CLL, increased number of platelets is an important prothrombotic factor, to which the presence of metabolic syndrome components adds. In advanced stages, increased mean platelet volume and chemotherapy are the main thrombotic risk factors, to which one can add the decrease in cholesterol and triglyceride levels, which is associated with an increased mean platelet volume.

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COAGULATION FATOR XIII TYR204PHE GENE POLYMORPHISM AND ISCHEMIC STROKE

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Background. A gene variant in the subunit A1 of the coagulation factor XIII (factor XIII A1 Tyr204Phe) was previously described and was strongly associated with ischemic stroke, with a 9-fold increased risk of ischemic stroke. The risk was further increased in carriers of Phe allele who were using oral contraceptives (20-fold). These results, however, have not been confirmed in other studies.**Aims.** To investigate the prevalence of the factor XIII A1 Tyr204Phe variant in a sample of Brazilian patients with ischemic stroke. **Methods.** 160 patients with a diagnosis of ischemic stroke referred to our service were included. All cases were tested for factor XIII A1 Tyr204Phe variant by polymerase chain reactions with fluorescent allele-specific oligonucleotide probes. Risk factors for ischemic stroke as obesity, hypertension, diabetes mellitus, current smoking, dyslipidemia and contraceptive use were also recorded. Exclusion criteria were cardioembolic events, malignancy or chronic inflammatory diseases. **Results.** The median age of the 160 patients was 37 years (range 15 - 72) and

105 (66%) were females. The frequency of acquired risk factors was: 4% obesity; 31% hypertension; 5% diabetes; 29% current smoking; 33% dyslipidemia; 25% (of females) contraceptive use. The factor XIII A1 Tyr204Phe variant was found in three patients (2%). **Conclusions.** This study shows a low prevalence of factor XIII A1 Tyr204Phe variant among Brazilian patients with ischemic stroke. A prevalence of this gene variant of 3.5% in Brazilian population had been previous described. Given its low prevalence, factor XIII A1 Tyr204Phe variant does not seem to be a major risk factor for ischemic stroke in this population.

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PROSPECTIVE EVALUATION OF THE PATIENT/NORMAL RATIO OF THE ACTIVATED PARTIAL THROMBOPLASTIN TIME AND DILUTED RUSSELL VIPER VENOM TIME IN LABORATORY DIAGNOSIS OF LUPUS ANTICOAGULANT (LA)

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Background. Lupus anticoagulant (LA) is clinically the most relevant among all antiphospholipid antibody tests. We recently reported a retrospective evaluation of the value of activated thromboplastin time and diluted russell viper venom time ratios (APTTr and dRVVTr) in a subgroup of samples negative for LA and with borderline mixing tests (8TH Meeting of the European Forum on Antiphospohilipid Antibodies). We suggest performing in these samples the neutralization tests. **Aims.** 1- To calculate the APTTr and dRVVTr in samples received to determine the LA. 2- To perform the neutralization test in selected samples negative for LA but with borderline mixing studies and high APTTr and dRVVTr.Design: Prospective observational. **Methods.** Patients n= 190; Samples: 195 (September 2011-January 2012). LA was done according to the recommendations of the Subcommittee on Lupus Anticoagulant/Anthiphospholipid Antibodies of the International Society on Thrombosis and Haemostasis.1) Screening tests: APTT using PTT-LA (STAGO) and dRVVT using Lac Screen (Instrumentation Laboratory)2) Mixing tests: pooled normal plasma homemade (1:1) proportion. Rosner Index (RI) and % of correction (%C) as criterion of mixing and confirmatory tests interpretation. Our cut off values: RI<0.12 and %C< 75 for APTT and dRVVT respectively.3) Ratios: cut-off value for APTTr >1.21 and dRVVTr >1.24.4) Confirmatory tests: homemade reagent for platelet neutralization procedure (PNP-APTT) and Lac confirm for dRVVT.Results. 24/195 (12.31%) were positive for LA .There were 5 samples with borderline correction in mixing tests and abnormal ratios for APTT (1) or dRVVT (4). We performed confirmatory tests to these samples and 3 of 5 samples were positive for LA increasing the final number of LA to 27.Conclusions: 1- The APTTr and dRVVTr as part of screening tests for LA can improve the diagnosis of LA in patients with negative LA, borderline mixing studies and positive neutralization tests. 2- This results must be confirmed with a larger number of samples.

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THE D DIMER TEST IN THE DIAGNOSIS OF VENOUS THROMBOEMBOLISM - AN AUDIT OF IT'S UTILIZATION IN AN IRISH TEACHING HOSPITAL AND A SURVEY OF JUNIOR DOCTOR KNOWLEDGE OF IT'S USES AND LIMITATIONS

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Background. The use of D-dimer measurement in the clinical setting has been most extensively studied and validated for the exclusion of venous thromboembolism (VTE) in low risk populations and the diagnosis and monitoring of DIC. In our laboratory, the Innovance® assay is used. It is a particle-enhanced immunoturbidimetric assay for the quantitative determination of cross-linked fibrin degradation products. D Dimer assays are widely used in Irish hospitals. There are many reasons for D Dimers to be elevated and if this test is not judiciously used, patients may undergo unnecessary radiological procedures. **Aims.** D-Dimer tests were reviewed prospectively to ascertain: 1. Appropriateness of the test 2. Accuracy of the test. Junior doctors were surveyed to see if there were any obvious knowledge gaps that could be filled by a teaching session to improve the institutions use of the test. **Methods.** The audit was carried out between February 15th and march 14th 2011. Consecutive D Dimer tests were reviewed by obtaining the specimen numbers from the analyzer and reviewing the medical notes of those tested. Junior doctors were also given a survey to fill out to ascertain their knowledge of the indications for performing a D-Dimer test, how to interpret the results and the cost of the test. **Results.** 345 cases were reviewed. The average age of those tested was 58.91 years (Range 14-95). 156 (45%) of those tested were men. Most tests were requested in the emergency department. The main reasons for requesting the test were dyspnoea, chest pain, general decline and limb swelling but it was requested for a wide variety of reasons, many of which were inappropriate. 193 patients had positive D dimer results and 12 of these had a proven thrombus. The high level of positive tests did not lead to a correspondingly high level of radiological procedures. 31% of the junior doctors in our institution responded to the survey, and their overall knowledge of the use of the test was good. 60% recognized that the D Dimer test is useful to out rule VTE when there is a low suspicion and 76% agreed that the test was most useful for patients with a low probability of VTE where a negative result makes it unlikely that they have VTE. 92% felt that a risk assessment score e.g. Wells score was required in most situations. Only 2 respondents felt that it was a good screening test if it was unclear what was wrong with the patient. Only 4 respondents had read the emergency medicine handbook D Dimer section. **Conclusions.** The D Dimer test is a useful test for out-ruling VTE. The limitations of the test, specifically those of the assay used by the institution must be fully understood by those ordering it. The greatest yield is obtained when the test is used in accordance with guidelines.

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IMPACT OF THROMBOPHILIA ON RISK OF PERINATAL STROKE IN NEONATES: A RETROSPECTIVE STUDY IN 54 CASES

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Background. Perinatal stroke (PS) may be defined as an acute neurologic syndrome with chronic sequelae due to cerebral injury of vascular origin occurring between 20 weeks gestation and 28 days postnatal life. There is increasing evidence of role of inherited thrombophilic factors in pathogenesis and clinical outcome of PS. **Aims.** The aim of this study is to value the role of inherited thrombophilia observed in neonates with stroke. **Methods.** 141 patients with diagnosis of PS or presumed PS were admitted to the Pediatric Hospital Bambino Gesù of Rome from 2001 to 2010. Assesment of thrombophilia was available only in 54 patients (26 females and 28 males), so the our analysis was performed only in this cohort. The mean age at presentation was 8 months (range birth-7 year); 9 patients (16.6%) were diagnosed at birth, 43 children (79.6%) were diagnosed as presumed PS. The mean follow-up was 64 months. In the patients with presumed PS, focal neurological signs (hemiparesis, epilepsy, psychomotor delay) were the most common presentation. In all patients arterial infarction was documented by MRI, performed at the admission to the hospital. In 7 patients familiar history for thrombophilia (1), cerebrovascular event (2) or epilepsy (4) were observed. At the last follow-up, 6 patients did not presented disabilities; 29 patients had only emisyndrome, 9 had cognitive and motor impairment, 10 had motor deficits and epilepsy, that in 3 patients was uncontrolled by pharmacological therapy. Nobody showed recurrence of the stroke, or died. Of 48 patients with persistent neurological disabilities, 22 (45.8%) had inherited thrombophilia: 9 had factor V Leiden, 3 prothrombin II and 10 MTHFR; when we limited the observation to children with PS at the birth, 5/9 patient had a mutation, that was in 3 children factor V Leiden and in a patient it was observed the presence of Factor V Leiden and prothrombin II G20210A. The other child had MTHFR. In the patients without disabilities inherited thrombophilia was observed in 3 children. There was not significative correlation between thrombophilia and the occurrence of stroke or clinical outcome*(p 0.12)*.However when we considered only patients with PS at the birth, the presence of inherited thrombophilia resulted significative*(p 0.039)*, but it did not influence clinical outcome*(p 0.16)*. **Results.** Thrombophilia was documented in 45.8% of the children. In our study we did not find a significative correlation between the thrombophilia and the stroke in patients with presumed PS. **Conclusions.** The datas observed in patients with PS need to be confirmed, because of the limited number of patients and the retrospective analysis. Prospective observational study could be useful to assess the role of inherited thrombophilia in PS and the safety of eventual anticoagulant prophilaxys in neonates with high risk of PS or recurrence of thromboembolism. For that we believe that thrombophilia testing should be performed on any child with PS and their parents. to reduce the risk of PS in future life of children and in eventual pregnancies of mothers.

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HYPERCOAGULABLE STATE; PARADIGM SHIFT IN CLINICAL STATUS OF THALASSEMIA MAJOR PATIENTS

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Background. Customary blood transfusion programs and iron chelation remedies have dramatically extended the life expectancy of thalassemia major cases into fourth decade of life. Morbidity due to the diverse siderotic and non siderotic complications significantly obliterates the worth of life. Hypercoagulopathy and thromboembolic manifestations are being increasingly acknowledged in transfusion dependent thalassemics. Preliminary studies in last two decades have shown that hemostatic amendment obligate thalassemic at the verge of thromboembolism. We accomplished this study to determine if this might be owing to the deficiency of naturally occurring anticoagulants; protein C, protein S and antithrombin III. **Aims.** The aim of this study is to evaluate the deficiency of coagulation inhibitors in Pakistani thalassemic individuals, so that timely intervention could be done to secure the life of high risk patients. **Methods.** This is a prospective cross sectional study, started from August 2011 to Jan 2012. Thirty patients with beta thalassemia major were enrolled in this study. Complete blood counts, liver function test, serum ferritin and hepatitis B and hepatitis C were analyzed by standard laboratory methodology. Hemostatic markers includes prothrombin time, activated partial thromboplastin time, protein C, protein S and antithrombin III which were analyzed by CA 1500 (Sysmex Japan). We computed frequencies for isolated and grouped deficiencies, and percentages for the prevalence of Hepatitis B and C. We also computed spearman correlation at 5% level of significance to identify relationship between the deficiency of protein C, protein S and antithrombin III with hemostatic markers and maternal characteristics. **Results.** 30 patients incorporated in our study having mean age 13.1±5.4 years. Male to female ratio is 1:1. None of the patient was splenectomized. The mean pre-transfusion hemoglobin was 7.84±1.42 g/dl. Prolonged prothrombin time was accredited in 17% patients while prolongation of activated partial thromboplastin time was endorsed in 13%. Serum ferritin was considerably high with the mean of 4300.3±2435.8 ng/ml. The prevalence of hepatitis C is substantially higher (33%) as compared to hepatitis B (3%), however, its relationship with prothrombotic markers could not be established statistically. Protein C was predominantly deficient in 60% patients whereas protein S in 37% and antithrombin III in 27%. Protein C deficiency was exceedingly distinctive and divulges positive correlation with anemia, thrombocytopenia, prolonged PT and high ferritin. The proportions of all these deficiencies were higher in cases above 10 years of age. **Conclusions**. Our study revealed substantial decrement of the prothrombotic markers predominantly protein C, which may be implicated for elevated incidence of thrombotic events in thalassemia patients. These outcome raise the concern either it will be cost beneficial to suggests prophylactic anticoagulation, particularly for those who are at high risk and should all the individuals with thalassemia needs screening for the prothrombotic markers. Large scale prospective studies will be required to delineate specific recommendations for screening and the circumstance when to start prophylactic therapy and upon which point of deficiency.

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MEDIUM PLATELET VOLUME - AN IMPORTANT PREDICTIVE FACTOR FOR AGGRESSIVE EVOLUTION IN ACUTE MYOCARDIAL INFARCTION PATIENTS

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Background. Larger platelet are hyperreactive and represent a risk factor for thrombotic complications, like acute myocardial infarction (AMI) or stroke. A**im** of the present study was to determine the importance of platelet indices in risk stratification of AMI patients. **Methods.** We performed the medium platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) in 55 consecutive AMI patients (30 STEMI and 25 non-STEMI) admitted in our clinic during 1 Oct. - 31 Dec. 2011, splitted in groups using variables like heart failure (HF) presence, association of diabetes mellitus or hypertension (HTA), iterative thrombosis in medical history. **Results.** MPV is increased in female patients with history of thrombosis vs. female patients without thrombosis with history of thrombosis as follows: 7.4 (7.13-11.17) vs. 6.98 (6.152-7.323), p=0.01, patients with HF and HTA, vs. patients with HF without HTA 8.07 (6.82-13.35) vs. 6.85 (6.09-7.32), p=0.02 or HF and age over 65 7.27 (6.80-7.67) vs. age below 65 7.52 (7.12-8.65), p=0.01. Inside the STEMI = 65 years group of patients, MPV was higher than non-STEMI group patients = 65 years, p=0.03. We did not find any significant differences for MPV in diabetes mellitus group. PDW is also increased in HF and = 65 years group vs. HF and <65 years group, median: 18.3 (16.98-19.61) vs 17.2 (16.72-17.4), p=0.01. There are not any differences for PDW or PCT between STEMI and non-STEMI groups. The analysis of risk factors in = 65 years patients showed 75% for non-STEMI group and 40% for STEMI group; actually, non-STEMI was more frequent in elderly patients, OR 0.22. p=0.02. The incidence of either non-STEMI or STEMI was not influenced by diabetes, hypertension, thrombosis history, cardiac failure. **Conclusions**. MPV=7.4 is the most important risk factor in elderly STEMI patients, and also in heart failure and hypertension patients, or female patients with recurrent thrombosis, either AMI or stroke.

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METABOLIC SYNDROME, MEAN PLATELET VOLUME AND RISK OF THROMBOSIS IN PATIENTS WITH PHILADELPHIA NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASIAS

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Background. Philadelphia negative chronic myeloproliferative neoplasias predispose to thrombosis. Metabolic syndrome is involved in the activation / hyperreactivity of platelet. An increased mean platelet volume is known as an index of platelet activation and thrombotic risk. Hypertriglyceridemia - a component of metabolic syndrome - is associated with increased plasma levels of protrombotic markers (plasminogen activator inhibitor-1 and tissue plasminogen activator). **Aims.** We aimed to analyze the relationship between metabolic syndrome, mean platelet volume and thrombotic history of patients with chronic myeloproliferation. **Methods.** In the study were included all the 50 patients with Ph negative chronic myeloproliferative neoplasias who were hospitalized in 2011 in the Hematology Department of Emergency Clinical County Hospital of Sibiu and appear in the electronic filing system ATLAS. We studied: age, gender, diagnosis, platelet count, mean platelet volume, cholesterol, triglycerides, number of components of metabolic syndrome, treatment, thrombotic accidents. We note that blood samples were analyzed in the laboratory within 2 hours after withdrawing. We compared the data of patient with and without one or two components of the metabolic syndrome and those with and without a history of thrombotic accidents. Statistical analysis was performed with the arithmetic mean, standard deviation, Student t test and Pearson test. **Results.** The number of components of metabolic syndrome is directly correlated with serum triglyceride (r=0.454) and, slightly, with mean platelet volume (r=0.253). Of the 50 patients, 35 had at least one component of metabolic syndrome (group A) and 15 - none (group B). Patients in group A had an older age ($p<0.01$), higher number of platelets ($p=0.01$), higher cholesterol and triglycerides ($p<0.05$, respectively, $p=0.01$). From the entire group, 15 patients had at least two components of the metabolic syndrome (group C) and 15 - none (group B). Patients in group C had significantly higher cholesterol and triglycerides than those in group B ($p<0.05$, respectively, $p<0.005$). Among patients who had at least one component of metabolic syndrome, those with a history of thrombotic accidents had higher triglycerides values ($p <0.005$) and mean platelet volume ($p<0.0005$) than in those without thrombosis in the past. Among patients who had at least two components of the metabolic syndrome, those with a history of thrombotic events had higher triglycerides values ($p <0.05$) and mean platelet volume ($p <0.05$) than in those without thrombosis in the past. In the entire group the total number of antecedents of thrombotic events is directly associated with higher values of average platelet volume (r=0.470) and triglycerides (r=0.263). **Conclusions**. Among the components of metabolic syndrome, hypertriglyceridemia is mostly associated with the presence of thrombotic accidents in history. The fact that patients who had thrombotic stroke continues to have an increased platelet volume and that this is associated with the number of components of metabolic syndrome, means that they continue to be prone to produce thrombosis.

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EXPERIENCE WITH ENOXAPARIN IN PEDIATRIC DEEP VEIN THROMBOSIS: MONITORING OF TREATMENT

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Introduccion. The diagnosis of Deep Vein Thrombosis (DVT) in children has an estimated incidence of 0.7/10,000. It´s related with high hospitalization rates and central venous catheter (CVC) carriers. Early diagnosis and treatment are important because it is a serious disease that, if untreated, can lead to a postthrombotic sequel or a pulmonary thromboembolism (PTE). There is a little data on clinical outcomes following therapeutic low-molecular-weight heparin revealing problems in anti-Xa monitorization levels in children current recommendations for anticoagulant and therapy are based in the adult setting. **Aims.** To describe demographic and clinic characteristics of pediatric patients with DVT in our institution. To evaluate fluctuations in enoxaparine doses and anti-Xa concentration in patients until they achieve therapeutic levels. **Methods.** A retrospective chart review was performed using anti-Xa test laboratory data base in patients under 16 years old with diagnosis of DVT, treated with enoxaparin in our institution since January 2005 to February 2012. Data on enoxaparin doses, anti-Xa concentrations, clinical characteristics and outcomes were obtained. **Results.** In our cohort (n=14), thrombosis was diagnosed at a median age of 3.5 months (0.5-168),male/female ratio 8/4. Cerebral venous sinus thrombosis (CVST) was the most common indication (5), followed by DVT with/without PTE (4), thrombosis related to CVC (3), thrombosis related to vascular prosthesis (1) and purpura fulminans secondary to acquired protein S deficit (1). Systemic sepsis (9), CVC carriers (9), malignancies and cardiovascular interventions (2) were the most frequent risk factors. Genetic thrombophilia pattern was detected in 2 cases; one with FV Leiden who develop DVT with PTE associated to obesity and oral contraceptives use; one patient with thrombin mutation (G20210A) developed CVST related to asparaginase for ALL therapy. Enoxaparin was initiated at mean dose of 1.5 mg/kg/every 12 hours. Therapeutic anti-Xa concentrations (0.5-1 U/mL) were achieved in 12 patients (85%); 4 patients with doses of 1.3-1.5mg/kg/12 h, 6 patients with 2 mg/kg/12 h, one 14 days patient need 2.4 mg/kg/12 h and one 2 year patient need 2.7 mg/kg/12 h. The Mean of number controls were 3 (1-5), with a median of 11 (3-63) days to achieved therapeutic anti-Xa concentration. During maintenance therapy 2 patients lost therapeutic concentrations with secondary therapeutic levels after increasing dose. Early discontinuation of enoxaparin occurred in 1 patient secondary to cerebral hematoma by tumoral hemorrhage, 2 patients are still on therapy; in the rest of cases complete resolution were achieved. No mortality related to anticoagulant therapy was observed. **Conclusions**. It seems that current adult dose recommendation may not be appropriate for children; age based enoxaparin dose should be adapted in early pediatric patients. Anti-Xa concentrations fluctuate during maintenance therapy being necessary periodic anti-Xa controls. Despite this, enoxaparin seems efficacious in thrombosis resolution. Further studies to determine the role of anti-Xa concentrations for control on outcomes in this population are needed.

Platelets 2

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RESULTS FROM A PHASE IV OPEN-LABEL STUDY EVALUATING CHANGES IN BONE MARROW MORPHOLOGY IN ADULT IMMUNE THROMBOCYTOPENIA (ITP) PATIENTS RECEIVING ROMIPLOSTIM: ANALYSIS OF THE 1-YEAR ROMIPLOSTIM COHORT

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1. **Background.** Patients with ITP may have evidence of reticulin fibers in the bone marrow without apparent clinical sequelae. In clinical trials with the thrombopoietin mimetic romiplostim, 10 of 271 patients were reported to have increased reticulin fibers of various grades (1-4, grade 4 includes minimal collagen) in the bone marrow that often decreased in grade after romiplostim discontinuation (documented in 3 cases). However, bone marrow biopsies were not systematically performed in these trials.2. **Aims.** This study of adult ITP patients treated with romiplostim was designed to prospectively evaluate bone marrow biopsies for collagen and reticulin levels. Changes in cytokine levels (TGF-β, PDGF, bFGF, and osteoprotegerin) relative to bone marrow changes were also assessed.3. **Methods.** Eligible ITP patients had platelet counts <50x10^9/L, received ≥1 prior ITP therapies, and had no collagen fibrosis in baseline bone marrow biopsies (i.e., before romiplostim). Three cohorts of patients were scheduled to have biopsies after 1, 2, or 3 years of romiplostim, respectively, with romiplostim dosed to maintain platelet counts at 50-200x10^9/L. Bone marrow biopsies were also performed when patients discontinued early or failed to achieve or maintain a response to romiplostim, with lack of response defined as having platelet counts ≤20x10^9/L for 4 consecutive weeks at the maximum romiplostim dose of 10 µg/kg. An independent expert bone marrow panel reviewed bone marrow data provided by a central pathology laboratory.

Table 1-Year Romiplostim Cohort Results

Patients	Number
Enrolled	50
Had 1 year of romiplostim and biopsy	35
Missing 1 year biopsy	15
- Due to serious adverse event	1
- Withdrew consent	5[1]
- Nonresponders	4[2]
- Died	3
- Severe joint pain	1
- Discontinued due to investigator decision	1
Bone marrow biopsies	38[2]
Biopsies evaluable for collagen (trichrome stain)[3]	33
- Positive for collagen	0
Biopsies evaluable for reticulin (silver stain)[3]	32
- Increase in reticulin grade from grade 0 to 2	2

[1] One of the 5 were nonresponders
[2] Bone marrow biopsies were obtained from 3 of the nonresponding patients prior to the one year assessment.
[3] Trichrome and silver staining could not be performed on all 38 biopsies due to inadequate samples.

Fibrosis was measured using the modified Bauermeister scale (0 no reticulin, 1 occasional fine fibers/foci fine fiber network, 2 fine fiber network, 3 diffuse fiber network, scattered coarse fibers, 4 diffuse coarse fiber network with areas of collagenization). Collagen was detected by trichrome staining and reticulin by silver staining.4. Results. Of the 50 patients enrolled in the 1-year romiplostim cohort (54% female, mean age 55.5 years, range 20-86 years), 37 (74%) had grade 0 reticulin, 13 (26%) had grade 1 reticulin, and none had collagen in baseline biopsies (i.e., before romiplostim). Thirty-five patients completed one year of romiplostim; of the 15 discontinuing (details in Table), 3 (all nonresponders) also had bone marrow biopsies performed. Examination of the 38 available biopsies from the 1-year cohort showed that none had collagen and 2 had an increase in reticulin grade from 0 to 2. The safety profile was similar to that seen in previous trials. Overall, cytokine levels remained within normal levels. As there were few bone marrow findings, no correlation between cytokine levels and changes in bone marrow morphology were evident. There were no neutralizing antibodies to romiplostim or thrombopoietin. Three patients died (not attributed to romiplostim); causes were fungal sepsis in a patient with long-standing corticosteroid use, cerebral hemorrhage (platelet count 4 days prior was 8x10^9/L), and pulmonary hemorrhage (platelet count one day before death was 59.8x10^9/L).5. Summary/ **Conclusions.** There was a low incidence of increased reticulin and no cases of collagen formation in the 1-year romiplostim cohort, consistent with findings from previous romiplostim studies. This ongoing study will provide additional data regarding bone marrow changes with up to 3 years of romiplostim.

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STICKY PLATELET SYNDROME AND THE GENETIC VARIABILITY IN THE PLATELET GLYCOPROTEIN 6 GENE IN WOMEN WITH FETAL LOSSES

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Background. Sticky platelet syndrome (SPS) is the second most common hereditary thrombophilia after resistance to activated protein C (APC-R) and the most common thrombophilia associated with arterial thrombosis. Either after antiphospholipid syndrome is SPS the second most common thrombophilia that causes recurrent spontaneous abortions or fetal loss syndrome.**Aims.** The aim of the study was to evaluate the genetic variability of the GP6 gene in women with sticky platelet syndrome (SPS) type I or II and fetal losses. **Methods.** 27 women with SPS type I or II, clinically manifested as fetal loss, and 42 healthy women (blood donors) without SPS and with no previous history of fetal loss and venous or arterial thrombosis were enrolled. All subjects gave their informed consent with participation in the study. SPS was diagnosed by platelet aggregometry (PACKS-4 aggregometer, Helena Laboratories) according to the method and criteria described by Mammen and Bick. Seven single nucleotide polymorphisms (SNP) of the GP6 gene (rs1654410, rs1671153, rs1654419, rs11669150, rs1613662, rs12610286, rs1654431) were evaluated with the use of restriction fragment length polymorphism analysis.**Results.** We found a higher occurrence of three SNPs of GP6 gene in SPS group compared to controls (rs1671153, 0,204 vs. 0,048; OR 5,116; CI 1,536-17,03; rs1654419 0,204 vs. 0,071; OR 3,326; CI 1,149-9,619; rs1613662 0,204 vs. 0,071; OR 3,326; CI 1,149-9,619). The haplotype analysis showed a significantly higher occurrence (p<0,05) of two haplotypes in SPS women (CGATAG, 0,204 vs. 0,048; OR 4,961; CI 1,488-16,53 and CTGAG, 0,185 vs. 0,059; OR 3,568; CI 1,142-11,14, respectively). **Summary and Conclusions.** Our results, especially the higher occurrence of two GP6 haplotypes in SPS women with fetal losses, can support an idea that variability of the GP6 gene may be associated with the platelet hyperaggregability in these women.*Acknowledgement: This study was supported by the grant Vega 1/0029/11, project CEVYPET (ITMS 2622012053) and project CEPV II (ITMS 26220120036)*.

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ROLE OF RETICULATED PLATELETS IN THROMBOCYTOPENIC CHILDREN

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Background. Thrombocytopenia can result from production insufficiency of the bone marrow or increased peripheral destruction. Reticulated platelets (RP) are the youngest circulating platelets recently released from marrow megakaryocytes and contain abundant mRNA which can be quantified by flowcytometry. **Aims.** We aimed to evaluate RP % by flowcytometry in different types of thrombocytopenia, to find out their clinical significance and to correlate RP% with response to therapy. **Methods.** RP% was estimated by flowcytometry on peripheral blood of eighty patients suffering from thrombocytopenia(their ages ranged from1.3to15years) recruited from Hematology/Oncology Clinic, Children Hospital, Ain Shams University. They were twenty patients with acute immune thrombocytopenic purpura (ITP) (mean age 6.15 + 2.32 years), twenty chronic ITP patients (mean age 7.92 + 3.27 years), twenty patients with bone marrow failure (mean age 6.61 + 4.37 years) and twenty patients with thrombocytopenia secondary to chemotherapy (mean age 8.4 + 2.7 years). Twenty healthy age and sex matched subjects (mean age 6.75 + 2.84 years) were included as control group. **Results.** The study revealed significant increase in percentage of reticulated platelets in acute and chronic ITP patients when compared to control group, (P<0.0001, 0.04 respectively). In bone marrow failure group, the percentage of reticulated platelets was significantly higher than the control group (P <0.0001). In group of thrombocytopenia secondary to chemotherapy, there was no significant difference in percentage of reticulated platelets compared to control group (P>0.05). When chronic ITP patients were followed up six months after therapy they revealed significantly higher mean RP% in

patients with platelet count>100 x 10^9/L than those with platelet count<100x 10^9/L with positive correlation between RP% and platelet count (P<0.05). In patients with bone marrow failure, RP% was higher in patients whose platelets increment >30x10^9/L than those with platelets increment <30 x 10^9after therapy. **Conclusions.** Measurement of reticulated platelet percentage by flowcytometry is useful in classifying the origin of thrombocytopenia into peripheral or central. High RP% can predict patients with good response to therapy especially in patients with chronic ITP and bone marrow failure

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RITUXIMAB BEFORE SPLENECTOMY IN ADULTS WITH PRIMARY IDIOPATHIC THROMBOCYTOPENIC PURPURA: A META-ANALYSIS

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Primary immune thrombocytopenia (ITP) is an acquired immune-mediated disorder with absence of any underlying cause. Corticosteroids are the standard initial treatment. Splenectomy is the main second-line treatment. A trend to delay or avoid splenectomy has developed thanks to new agents like Rituximab. Few studies have assessed the response rate to Rituximab in chronic ITP. We performed the first meta-analysis of randomized clinical trials and observational studies on rituximab as an effective splenectomy-avoiding option in adult chronic ITP. Overall methods were adapted from MOOS and PRISMA guidelines for meta-analysis. Two hematologist investigators carried out study selection and data extraction independently, recording ORR and CR as primary assessment criteria. 364 records were identified through electronic databases. 19 retrospective or prospective observational studies were retained after removing duplicate studies and full-text analyses. For 368 non-splenectomized patients after rituximab ORR was 57 % (IC95: 48-65), CR was 41 % (IC95: 0.33-0.51) for 346 patients. Results were stable for ORR and CR in all subanalyses. In univariate or multivariate mixed-effect meta-regression, age was the most relevant effect. According to our results, rituximab should be used in second line in non-splenectomized patients.

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GAUCHER DISEASE: LONG-TERM FOLLOW UP AND COMPLICATIONS IN PATIENTS TREATED WITH ENZYME REPLACEMENT THERAPY (ERT) IN A SINGLE CENTRE

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Background. Gaucher disease (GD) is a common inherited lysosomal storage disorder due to an autosomal recessive defect of the gene encoding glucocerebrosidase enzyme, responsible for accumulation of glucosylceramide into reticuloendothelial cells. GD is a clinically heterogeneous disorder and it is classically classified in type 1 (non-neuronopathic disease), types 2 and 3 (acute and chronic neuronopathic disease, respectively). Actually, the standard therapy for GD is ERT with imiglucerase, but alternative therapies became available. **Aims.** To study the improvement of the haematological, visceral, biochemical and bone parameters during ERT in patients with GD in a long period of time. **Methods.** Twelve patients (pts) (9 males and 3 females) are followed for a mean of 8 ± 3 years at Hereditary Anemia Centre in Milan. In all patients GD type 1 was diagnosed by the enzymatic activity dosage and the molecular analysis. A yearly follow up was performed, evaluating biochemical parameters (liver and kidney function tests, coagulation tests, count of blood cells (CBC), chitotriosidase levels, vitamine B12 and folic acid dosage, iron balance tests), liver and spleen diameters (US of the abdomen), cardiac and pulmonary function (ECG, echocardiographic examination, chest x-ray, functional respiratory tests) and bone involvement (dual-energy X-ray absorptiometry-DEXA-, magnetic resonance-MRI- of pelvic track and femurs). GAUSSI-I scores were calculated to describe the burden of GD. Each parameter was evaluated before starting ERT and in 2011. The mean actual dose of ERT was 31 ± 9 UI/kg every two weeks. The mean period of treatment was 10 ± 6 years. **Results.** The mean age of our patients was 43 ± 13 years. The genotypes represented were N370S/N370S homozygous (3 pts), N370S/L444P (3 pts), N370S/P401L (1 pts), N370S/IVS2+1 (1 pts), N370S/delta55 (1 pts), N370S/RecNcil (1 pts), N370S/unknown (1 pts), unknown (1 pts). Thrombocytopenia and splenomegaly were the most frequent presenting symptoms and all patients had skeletal involvement at diagnosis. During the follow up a significant improvement of platelets (PLT) (p = 0,0461) and a significant decrease of spleen diameter (p = 0,086) were detected; a good improvement of haemoglobin (Hb) and a good reduction of GAUSSI-I were observed also if they were not statistically significant (Figure 1). During the first year of ERT there is an improvement of levels of Hb and PLT and a decrease of spleen diameter. Vertebral and femoral Z score improvement (p = 0,0002 and 0,0024) appeared later in time. During the period of follow up one patient developed a myelodisplastic/myeloproliferative syndrome and a femoral necrosis. **Conclusions.** GD is a multiorgan and a multidisciplinary disease. Its early diagnosis is necessary to start as soon as possible the treatment, reducing the invalidating complications. This study showed that the administration of an adeguate and personalized ERT improves not only the haematological and visceral parameters in a short period of time but also the bone mineral density in a longer period. A disease severity score is useful to quantify the response of the disease to the treatment. Long term complications of GD, such as haematological malignant diseases, has to be considered.

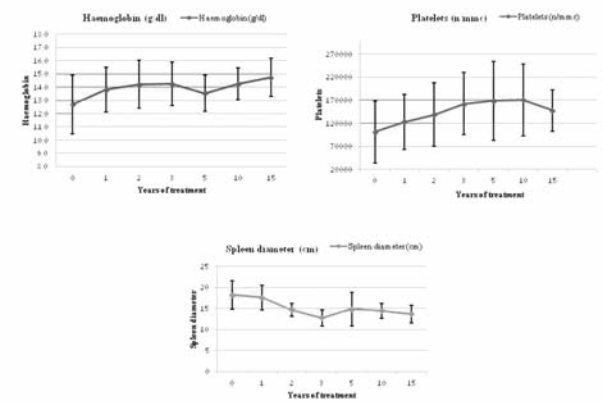

Figure 1: Haemoglobin levels, platelets count and spleen diameter during ERT treatment

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INTERACTION BETWEEN T AND B LYMPHOCYTES AND THE IMPACT OF PROLIFERATION OF B LYMPHOCYTES ON THE PATHOGENESIS OF IMMUN THROMBOCYTOPENIA IN CHILDHOOD

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Immune thrombocytopenia (ITP) is a disease characterized by forming autoantibodies against antigens on platelets and the clearance of these antibody coated platelets by reticuloendothelial system. The aim of this study is to determine the levels of B cell activating factor (BAFF) and a proliferation inducing ligand (APRIL), the interaction between T and B lymphocytes, the impact of proliferation of B lymphocytes in the pathogenesis, and other cellular interactions probably having influence on this proliferation (T lymphocyte, activating B lymphocyte with complement, relationship between the levels of BAFF and APRIL, and the expressions of CD 21, CD40, CD154) in ITP of childhood.Twenty acute ITP patients, 20 chronic ITP patients, and 20 healthy subjects as a control group were enrolled in this study. BAFF, APRIL, IL-4, and IFN- γ in sera by ELISA, expressions of CD19, CD 3, CD21, CD40, CD154 inleucocytes and lymphocytes by flow cytometry were measured in both patient and healthy groups. We also measured these parameters again after the therapy between the day seven and fourteen.Informed consent from all participants was obtained for this study.There was no difference between groups for gender. Median age was 86,5 ± 50,3 months (range: 16-204 months) within whole study group without any significant difference among groups. Platelet levels were lower in acute and chronic ITP groups before therapy compared with controls. But there was no difference between groups for any other hemogram parameters. Sixteen patients in acute ITP group were treated with IV immunglobulin and four with high dose methylprednisolone. In chronic ITP group, nine patients were treated with IV immunglobulin and three were treated with high dose methylprednisolone. Twenty one patients (fourteen acute and seven chronic) were studied again after therapy between the day seven and fourteen.Serum BAFF levels, evaluated for the impact on the pathogenesis of ITP in childhood, were significantly higher in acute ITP group than that of both chronic ITP and healthy controls and were significantly decreased compared with pretherapy. APRIL, second molecule evaluated for the impact on the pathogenesis of İTP in childhood, which is homologue to BAFF, were not correlated with BAFF levels and similar in groups before and after therapy. IL-4 and IFN- γ levels, determining the Th1 and Th2 response, were similar in groups before and after therapy.

Expressions of CD3, CD19, CD21, CD40, and CD 154, evaluated for the interaction between T and B lymphocytes, were also similar in groups before and after therapy. Although the number of patients in this study is limited, this study shows that BAFF plays role in the pathogenesis of acute ITP, and data from adult ITP studies are not similar to data from childhood ITP. More comprehensive prospective studies are required for childhood ITP.

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CHILDHOOD IMMUNE THROMBOCYTOPENIC PURPURA: LONG-TERM FOLLOW-UP DATA EVALUATED BY THE CRITERIA OF INTERNATIONAL WORKING GROUP OF IMMUNE THROMBOCYTOPENIC PURPURA

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Background. Immune thrombocytopenic purpura (ITP) is a common bleeding disorder in childhood, characterized by isolated thrombocytopenia. The definitions, and clinical criteria used in the diagnosis and management of ITP are widely heterogeneous in different studies. International Working Group (IWG) of ITP has recently published a consensus report about standardization of terminology, definitions and outcome criteria in ITP to overcome these difficulties. The aim and procedure: The records of patients were retrospectively collected from January 2000 to December 2009. Our aim was to evaluate the data of children with ITP by using the new definition of IWG. **Results.** Thrombocytopenia less than 100x109/L were found in 201 children. The median follow-up period was 22 months (12-131 months). The median age and platelet count at presentation were 69 months (6 months-208 months) and 19x109/L (1-93x109/L), respectively. The female and male ratio was 1.16. We found two risk factors for chronic course of ITP; female gender (OR=2.55, CI=1.31-4.95) and age being more than 10 years (OR=3.0, CI=1.5-5.98). Life-threating bleeding occurred in 5% (n=9) of the patients. Splenectomy was required in 7 (3%) cases. Two of them was splenectomized within one year and the rest (n=5) had splenectomy after one year. When we excude splenectomized cases, CR was achieved in 139 out of 199 (70%) children at one year. The disease was also resolved in 9 (5%; n=9/194) more children from 12 to 90 months, achieving 76% (n=148/194) CR rate. **Conclusions**. In total, CR rates at 12 and 90 months has reached to 70% and 76% (n:148/194), respectively. Therefore, long-term follow-up is necessary in these children. Female gender and being more than 10 years old significantly influenced the chronicity.

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COST-CONSEQUENCE ANALYSIS COMPARING ROMIPLOSTIM TO RITUXIMAB IN THE TREATMENT OF ADULT PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN GERMANY

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Background. ITP is an autoimmune disorder characterized by isolated thrombocytopenia. Patients can experience a range of bleeding symptoms, some of which may require hospitalization and emergency treatment. Romiplostim stimulates platelet production via the thrombopoetin-receptor and is used for pretreated patients with chronic ITP. Other treatment options in this setting include off-label use of the immunosuppressant rituximab.**Aims.** This analysis assessed the cost per responder of romiplostim compared to that of rituximab in adult ITP patients in Germany. **Methods.** A decision analytic model was developed to estimate the 6-month cost per patient responding to treatment (achieving a platelet count ≥50x10^9/L). Romiplostim patients received weekly administrations; rituximab patients received a course of 4 weekly intravenous infusions. Resource utilization was based on international treatment guidelines and validated by clinical expert opinion. Unit costs were derived from German reimbursement lists, and included the costs of routine physician visits, treatment administration, and emergency care. Non-responders incurred the additional cost of rescue therapy (IVIg and prednisone) and hospitalizations/physician visits associated with bleeding-related events.**Results.** Response rates were 83% and 62.5%, as per most robust evidence, the romiplostim pivotal trials and systematic review on rituximab, respectively (Kuter et al, Lancet, 2008; Arnold et al, Ann Intern Med, 2007). Romiplostim and rituximab were associated with comparable treatment costs; mean overall 6-month cost per patient was €21,650 for romiplostim and €21,493 for rituximab. Dividing mean cost per patient by response rates yielded a cost per response of €26,084 for romiplostim and €34,389 for rituximab. The 32% increase in cost per platelet response observed for rituximab was mainly driven by the higher rate of rituximab non-responders, which increased the incidence of bleeding, and subsequently rescue medication use. Sensitivity analyses based on resource use, rescue medication use, and rituximab schedule were performed. **Conclusions**. In adult ITP patients in Germany, romiplostim was associated with lower cost per response over 6 months compared to rituximab. As romiplostim has a higher response rate, thereby lowering the incidence of rescue treatment usage, it represents a more efficient use of resources in the German healthcare system compared to rituximab. Although this analysis was not based on a head-to-head comparison of the two treatments, findings closely reflect the cost of treating ITP in clinical practice.

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BONE MARROW CD20 AND MORPHOLOGY IN CHILDREN WITH CHRONIC ITP

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Background. The presence of excess CD20B- lymphocytes in the bone marrow is related to an unfavorable outcome and anti-$CD20^+$ monoclonal antibodies therapy has been reported to be useful in patients with refractory ITP. **Aims.** To assess the bone marrow (BM) CD20 B-lymphocytes and BM morphology in relation to various clinical parameters and response to treatment in patients with chronic ITP. **Methods.** This prospective study was conducted on 30 patients with persistent thrombocytopenia >12 months after diagnosis of ITP. They were either non-responder or failed to maintain response to at least two treatment modalities before enrollment. All patients discontinued any medication 2 weeks prior to study entry. Any patient with life-threatening bleedings was excluded. Data collected included; age, sex, duration of illness, bleeding score and response to treatment. Laboratory investigations included; CBC, BM biopsy stained with reticulin to detect fibrosis and BM aspirate stained according to Leishman to assess plasma cells (%) , eosinophils (%), megakaryocytes (form and number/LPF). The number of megakaryocytes was rated as either normal (1 megakaryocyte per 1 to 3 low-power fields), increased (more than 2 megakaryocytes per low-power field), or decreased (1 megakaryocyte per 5 to 10 low-power fields). Assessment of CD20 B-lymphocytes was done by EPICS XL Coulter flowcytometer. Patients were considered positive for CD20 when >20% of total marrow lymphocytes expressed it. Informed consent was signed by parents, patients signed either ascent or consent forms. **Results.** Patients were 20 males (66.7%) and 10 (33.3%) females, their ages ranged from 2 -18 years old with mean age of 9.2 ± 4.1 years. The duration of illness ranged from 1- 9 years with mean duration of 3.5 ± 2.1 years. Mean platelet count at the study entry was 47.8 ± 31.5(x 10^3/ µl). Seventeen patients (56.7%) had normal level of CD20 B-lymphocytes (<20%), while 13 patients (43.3%) had positive CD20B-lymphocytes (>20%) with significantly higher bleeding score (p=0.025), lower mean platelets (p=0.026), a significant inverse correlation with platelet count (r=-0.48, p=0.008) and NR to steroids and Anti-D (p=0.035 and 0.04 respectively). $CD20^-$ patients showed significantly higher CR and PR to steroids (p<0.05). CD 20 B-lymphocytes level was not significantly related to age, gender or disease duration (p>0.05). All patients had normal bone marrow plasma cells (%), while bone marrow eosinophils were increased in all patients (4-13% with a mean of 6.4 ± 2.1%).There was an increased megakaryocytes number (3-8/LPF). No significant difference in CD20 B-lymphocytes level in relation to megakaryocytes (number or form) or eosinophils(%) (p>0.05). Fibrosis grade 1 to 2 was reported in two patients. Small sample size and unavailable BM samples at diagnosis were the 2 main limitations of this study. **Conclusions.** Increased bone marrow CD20B-lymphocytes is independent of bone marrow morphology and is related to lower platelet count, increased bleeding score and poorer response to steroids and Anti-D.

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NEW INSIGHTS INTO THROMBOPOIESIS IN NEONATAL SEPSIS

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Background. Thrombocytopenia is one of the most frequent hematologic abnormalities in the neonatal period, affecting about 18-35% of all patients admitted to the Neonatal Intensive Care Unit (NICU), with sepsis being among the most common causes of severe neonatal thrombocytopenia. It is unclear whether increased platelet consumption or decreased platelet production contributes to thrombocytopenia of septic neonates. **Aims.** To answer a question

whether thrombocytopenia in neonates with infection is a result of increased platelet destruction, or rather reflects decreased megakaryocytes due to sepsis-mediated suppression of megakaryocytopoiesis, we evaluated the effects of sepsis on neonatal thrombopoiesis, using a panel of tests. **Methods.** This prospective case-control study was conducted on 50 neonates with confirmed sepsis admitted to NICU at Pediatrics Department, Ain Shams University Hospitals. Thirty healthy newborns were included as controls. The enrolled neonates were subjected to detailed history taking, thorough clinical examination and laboratory investigations including complete blood count, C-reactive protein, blood cultures, and tests of thrombopoiesis; namely serum thrombopoietin (TPO) assay, flow cytometric analysis of reticulated platelets percentage (RP%) and calculation of absolute RP counts. **Results.** Septic neonates comprised 24 males and 26 females with mean gestational age of 36.0±3.1 weeks. Twenty-eight (56%) of the septic neonates were thrombocytopenic (platelets <150,000/μL). While platelets and RP counts were decreased, TPO and RP% were increased in septic neonates compared to healthy controls. Neonates with Gram-negative sepsis had the lowest platelet and RP counts and the highest TPO and RP% followed by those with fungal septicemia. Platelet counts showed inverse correlations with TPO and RP% and direct correlation with RP count. **Conclusions.** Neonates respond to sepsis by upregulating thrombopoiesis, where thrombocytopenia ensues when the rate of platelet consumption exceeds the rate of platelet production. Simultaneous measurements of serum TPO levels and RP% are helpful in discriminating hyperdestructive from hypoplastic thrombocytopenia among septic neonates.

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ASSESSMENT OF THE ANTIPLATELET ANTIBODIES(USING MACE 1: MODIFIED ANTIGEN CAPTURE) IN THROMBOCYTOPENIC HEPATITIS C VIRUS (HCV) CHRONICALLY INFECTED PATIENTS

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Background. Chronic HCV infection commonly manifest circulating autoantibodies and deposits of immune-complexes in various tissues outside the liver which may represent a major pathological pathway of extrahepatic manifestation development in the course of HCV infection. Several different mechanisms are reported to be related with thrombocytopenia in HCV positive patients: Portal hypertension and hypersplenism in end stage liver disease. Auto-immune reaction to platelets. Direct infection of platelets and megakaryocytes by HCV. In HCV-associated immune thrombocytopenia, the most common target reported was glycoprotein IIb/IIIa, but all other glycoproteins were also targets. **Design of the study.** Case control study. **Objectives of the study.** Assessment of serum level of Anti-platelet antibodies using **MACE 1** (Modified Antigen Capture)in HCV chronically infected thrombocytopenic patients. Detection of the presence of a correlation between the levels of the serum anti-platelet antibodies, platelet count and the viral load. **The study Population:** Case control study. *The study group:* 20 HCV positive thrombocytopenic patients. *Control Group*: 20 HCV positive non-thrombocytopenic patients. **Methods.** Medical histories, clinical examination, HCV- RT-PCR assay, liver functions, Anti-nuclear antibody, Rheumatoid factor. Anti-platelet antibodies using **MACE 1** (Modified Antigen Capture) is a qualitative solid phase ELISA designed to detect IgG antibodies to platelet GPIIb/IIIa. **Results.** There was statistically significant difference between the two group regarding the platelet count (101.5 ± 27.4 ×103 cell/ μl) in the patient group versus the control group (207 ± 49.5 ×103 cell/μl), (p< 0.05). Regarding the prevalence of anti-platelet antibodies a statistical significant increase among the thrombocytopenic patients 60%, compared to 10% in the control group (p< 0.05) with odds ratio. ALT, total and direct bilirubin and viral load are higher in the patient group compared to the control group. There was statistically significant correlation in the following: positive correlation between albumin (r= 0.5, p<0.05), and platelet count. There was a negative correlation between HCV-RT-PCR (r= - 0.4, p< 0.05) and platelet count. There were no statistically significant correlation for anti-platelet antibodies (r= -0.2, P= 0.4). reaching 13.5%. **Conclusions.** From the previously mentioned data, we concluded that HCV infection play an important role in the development of thrombocytopenia through production of PAIgG leading to platelet destruction together with the other contributing factors as it's an integrated process consisting of several factors which lead in the end to thrombocytopenia.

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PERSISTENT RESPONSE AFTER ROMIPLOSTIM DISCONTINUATION IN PATIENTS AFFECTED BY PRIMARY THROMBOCYTOPENIA

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Background. The efficacy of thrombopoietin mimetics (TPO-mimetics) in the treatment of chronic-refractory primary thrombocytopenia (pITP) patients (pts) has been demonstrated. The two available TPO-mimetics for the treatment of pITP are Romiplostim and Eltrombopag. These drugs have been approved by EMA for the treatment of chronic and refractory (post-splenectomy) pITP pts.It is well known that these drugs have to be chronically administered to maintain a platelet response.AIMS: To report our experience with Romiplostim treatment and discontinuation in chronic/refractory pITP patients. **Methods.** Twenty-three adult pITP pts [12 M, 11 F; median age, 59.3 yrs (31.9-79.8)] were treated with Romiplostim (initial dose 1μg/Kg/week). The dosage was adjusted in order to maintain the platelet count between 50-250x10^9/L. The median time between diagnosis and Romiplostim start was 14.4 yrs (0.08-38.75). All pts had already received ≥2 lines of therapy [median 2.5 (2-6)], which included prednisone, pulsed high-dose dexamethasone, immunoglobulins, rituximab, interferon, azathioprine and splenectomy in 8 pts. At the start of Romiplostim, the median platelet count was 11x10^9/L (2-32).RESULTS: Seventeen/23 (73%) pts reached a persistent response and 6/23 (27%) were non-responders. Four/17 (24%) responder pts maintained a persistent response after Romiplostim discontinuation. The characteristics of these latter 4 ptswere: median follow-up from therapy start, 20.5 months (12-34); median platelet count during therapy, maximum 382.5x10^9/L (406-1649), minimum 14x10^9/L^{-1} (1-79); median Romiplostim dose to achieve a response, 3.5 μg/Kg/week (1-4); median maintenance dose, 2.5 μg/Kg/week (1-3); median time to response, 3.5 weeks (1-5). Two out of these 4 pts started Romiplostim after 36.9 and 0.25 yrs from splenectomy, respectively; the first one started therapy after 38.75 yrs from the diagnosis of pITP and after 7 therapy lines; the second one started therapy after 2.3 yrs from pITP diagnosis and after 4 therapy lines. In an other pt, splenectomy was contraindicated because of a concomitant cardiovascular disease and age; she started Romiplostim after 14 yrs from pITP diagnosis and after 2 therapy lines; the 4th pt started Romiplostim after only 1 month from pITP diagnosis and prednisone treatment, because of the need to quickly increase the platelet count during a severe GI bleeding refractory to standard emergency therapy (high-dose immunoglobulins, platelet transfusions). All pts were receiving prednisone at the start of Romiplostim; all of them discontinued it at a median time of 14 weeks after response (5-21). They are persistent responders after 17, 9, 10 and 21 months after stopping Romiplostim, respectively. No serious adverse events were observed during and after therapy discontinuation. **Conclusions.** Four/23 (17%) pITP pts treated with Romiplostim maintained a persistent response after therapy discontinuation. So far, few literature data are available on the persistent response after TPO-mimetics discontinuation in pITP pts. Our experience could open new modalities in the use of these drugs, although randomized/controlled clinical trials are necessary to conclusively confirm these results.

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ACTIVATION AND FUNCTION OF ELTROMBOPAG-INDUCED PLATELETS COMPARED TO PLATELET FUNCTION IN CORTICOSTEROID- TREATED AND UNTREATED ITP PATIENTS

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Background. Eltrombopag is approved for the treatment of chronic immune thrombocytopenia (ITP). So far, no systematical analysis of platelet function in eltrombopag-treated ITP patients in comparison to corticosteroid-treated or untreated ITP patients has been performed.**Aims.** We aimed to assess a possible influence of eltrombopag on platelet function *in vivo. Patients/* **Methods.** Platelet function in chronic ITP patients after eltrombopag-induced platelet rise (group 1; n=10) was compared to a control group (group 2; n=12) of patients on continuous therapy with corticosteroids and untreated ITP patients in a non-randomized prospective single-center study. All patients gave written informed consent.Platelet function was assessed both, naive and after in vitro addition of agonists at 2 different time points and averaged. Platelet adhesion under high shear conditions (surface coverage, SC), P-selectin expression, and formation of platelet-monocyte aggregates (PMA) served as parameters of platelet function.Data are given as median [1st-3rd quartile]. Group 1 is listed first. In case of normally distributed data, the Student's t- tests and in case of non-normally distributed parameters the nonparametric Mann-Whitney U test were used to compare the groups. **Results.** Eleven patients (female=9) were included in the treatment group with eltrombopag (group 1). The control group (group 2) con-

sisted of 6 (female=4) patients on ongoing corticosteroid treatment and 6 untreated (female=5) patients. One eltrombopag-treated patient was not included in the final comparative platelet function analysis, because he had no treatment induced platelet rise. None of the included patients developed severe bleeding during the study period, and none received rescue medication. Two patients developed venous thromboses during eltrombopag treatment.Platelet counts [10^9/L] were 49.5 [45.00-59.00] in group 1 after eltrombopag-induced platelet rise, and 70.0 [64.0-80.0] in the control group. Naïve SC showed no difference between eltrombopag-treated patients and the control group with median levels of 5.80 [1.80-9.00] and 6.23 [5.03-11.75], respectively (p=0.488). Neither did activation with ADP (1.55 [1.05-2.70] and 1.80 [1.25-2.35]; p=0.692) or collagen (2.15 [1.90-2.30] and 2.75 [1.30-3.70]; p=0.339) reveal any difference in platelet activatability. Still, SC after addition of TRAP was decreased in the eltrombopag treatment group (1.00 [0.60-2.95] and 2.63 [1.90-4.48]; p=0.032).There were no differences in P-selectin expression [GeoMFI], neither without nor with addition of ADP, TRAP or collagen. The formation of PMA with and without agonistic stimulation was comparable in both groups, in the naïve measurement. Median levels after addition of ADP were 18.01 [10.15-53.13] and 27.23 [15.64-34.05] (p=0.489), and 47.24 [32.24-58.57] and 29.18 [21.84-44.09] (p=0.070) after addition of TRAP. **Conclusions.** We proofed a good functional competence of eltrombopag-induced platelets. No substantial hyperreactivity or increased activatability of eltrombopag-induced platelets was identified in comparison to platelets of untreated or corticosteroid-treated chronic ITP patients.
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LONG TERM FOLLOW UP ANALYSIS FOLLOWING RITUXIMAB SALVAGE THERAPY IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background. Several studies previously indicated the short to mid-term therapeutic activity of Rituximab in adults with relapsed-refractory ITP.**Aims.** In order to investigate the long term outcome, we retrospectively analyzed patients with ITP who were treated with Rituximab in our institution from October 1999 to March 2011. **Methods.** According to the different period of therapy, patients received either standard dose (SD) Rituximab (<i*e.* 375 mg/m^2 weekly for 4 weeks) or low dose (LD) Rituximab (<i*e.* 100 mg total dose weekly for 4 weeks). Primary objective was to evaluate EFS which included, as negative events, the absence of response within 2 months from Rituximab start and the lost of response. **Results.** 57 consecutive adult patients, median age 47 years (range 14-80), median platelet count 23 x 10^9/L were evaluated. All patients had active disease that had relapsed or was refractory to at least one full course of steroid therapy or was steroid dependent. Other treatments before Rituximab included splenectomy (3 patients), intravenous immunoglobulin (25 patients), azathioprine or cyclosporin-A (7 patients). The median time from diagnosis to Rituximab was 24 months (range 2-324). Rituximab was administered at SD in 32 patients and at LD in 25. Patients who received SD *vs.* LD Rituximab had better outcome as far as short term response (OR 66% *vs.* 52%, CR 50% *vs.* 28%), relapse rate (38% *vs.* 54%), 4 years EFS (40% *vs.* 24%). The only factor which correlated with EFS was the duration of the interval from diagnosis to Rituximab treatment. Three patients developed short term adverse events, 2 serum syndromes and 1 interstitial pneumonia. Six patients developed delayed adverse events including 4 neoplasms and 2 herpes zoster reactivation; 1 patient died for cerebral bleeding. During this period of observation no cases of opportunistic infections, progressive multifocal leukoencephalopathy or other severe infectious complications were observed.**Conclusions:** Rituximab SD appears a safe and active agent allowing in nearly 40% of cases to achieve long-term response. This effect seems to be superior when treatment is anticipated, thus suggesting a possible advantage of Rituximab treatment in an earlier phase of the disease.

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FACTORS ASSOCIATED WITH RESPONSE TO RITUXIMAB; ANALYSIS OF AN IRISH MULTI-CENTER STUDY OF RELAPSED OR REFRACTORY IMMUNE THROMBOCYTOPENIA (ITP)

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Background. In the treatment of relapsed or refractory ITP, rituximab is associated with response rates of 60%. The response is transient and 85% of patients will require salvage therapy within two years. Factors predicting a sustained response to rituximab are unknown. **Aims.** This retrospective multi-center study aimed to identify factors associated with a sustained response to rituximab. **Methods.** Patients who received rituximab for the treatment of relapsed or refractory ITP were identified from hospital records at 11 Irish haematology centers.Data were collected from patient notes and laboratory records.Response was graded as follows:1 Complete response. Platelet count > 1002 Good response. Platelet count 50-80 and > 50% increase from baseline platelet count3 Intermediate response. Platelet count 30-50 and > 50% increase from baseline4 No response. Platelet count stable or rise not meeting criteria for intermediate response5 Loss of response. Platelet count < 30 in a patient previously meeting criteria for response or need for salvage therapy.Overall response rate included Grade 1, 2 and 3 responses. **Results.** 117 patients with relapsed or refractory ITP were identified. No data on response were available for 12 patients and they were excluded from analysis.Of the 105 patients for analysis, 41.1 % were male and 58.9 % were female. The median age at rituximab treatment was 55 years. 85.7 % had primary ITP. Patients received a median of two prior treatment modalities, with 9.5 % having four or more treatments modalities. 20 % had undergone splenectomy.The median platelet count commencing treatment was 19x10^9/L. 97.8% of patients received rituximab at a dose of 375mg/m^2 weekly for four weeks.The overall response rate was 57.3% at one month with responses sustained for up to 36 months. There was no statistically significant difference in response rates when groups were compared for gender, age, number of prior treatments or use of steroid with rituximab.A difference in overall response was observed between splenectomised and non-splenectomised patients (42.1% versus 59.5% p=0.2) at one month and at six months (50% versus 64.9% p=0.38). The difference in response rate achieved statistical significance at 12 months (30.8% versus 58.6% p=0.05), at 24 months (11.1% versus 58.6% p=0.02) and at 36 months (16.6% versus 76.5% p=0.018). **Conclusions.** Rituximab offers an efficacious therapeutic option for treatment of relapsed or refractory ITP. Our study found older adults are as likely to respond to rituximab as younger patients.Non-splenectomised patients respond to rituximab and the response is sustained for up to three years. Randomised data are needed to determine the optimal treatment for young surgically fit patients with ITP.In patients who relapse following splenectomy, the response rate is lower and responses are not sustained. Thrombopoeitin receptor agonists may be more appropriate therapy for splenectomised patients requiring further ITP treatment.

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INVESTIGATION OF IL1B, IL1RN, IL4, IL6 AND IL10 GENE POLYMORPHISM IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA

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Background. The immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by the presence of autoantibodies against the platelet membrane glicoproteins. The inflammatory reaction is regulated by a balance

between pro and anti-inflammatory cytokines and it was reported that there was an association between the cytokine gene polymorphisms affecting the cytokine production and its secretion in autoimmune and malignant diseases both at the stage of formation, in the course of disease and their responses to treatment. **Aims.** In this study, the aim was to investigate whether there were any differences between the ITP patient group and the healthy control group in a population of the southwestern state of São Paulo, Brazil in cytokines gene polymorphisms *IL1B, IL1RN, IL4, IL6, IL10* known to be related to autoimmunity inflammation and to investigate the association between the identified genotypes and their responses to treatment. **Methods.** We investigated polymorphisms in genes using polymerase chain reaction, real-time PCR and polymerase chain reaction-restriction fragment length polymorphism **Methods.** Associations were evaluated using Fisher exact test. In total, 216 adult patients with ITP were matched with 118 controls and clinical data were obtained from medical records. **Results.** Analysis of allele and genotype frequencies of *IL1B*-511C/T, *IL1B*+3953C/T, *IL4* VNTR, *IL6*-174G/C, *IL10*-1082G/A, *IL10*-819C/T and *IL10*-592A/C polymorphisms showed no significant differences between the two groups. However, for the IL1RN VNTR polymorphism there was a significant difference between allele 2 frequencies, with genotypes non carriers of allele 2 significantly increased in ITP as compared to the controls (p <0.001; OR = 4.15, CI = 2.31 to 7.58). The same goes for the *IL4* -590C/T polymorphism in which there is an increase statistically significant of the C allele in ITP group. (p <0.001, OR = 0.42, CI = 0.29 to 0.6). Also, studying *IL10* haplotypes, the comparison between High Producer (GCC/GCC) and Middle (GCC/ACC, GCC/ATA) Lower (ACC/ACC, ACC/ATA, ATA/ATA) showed that patients with genotype High Producer are much more frequent in ITP (p=0,001; OD=0.008; CI =0 to 0,49). Analyzing the polymorphisms associated with clinical parameters, this study showed that *IL1B*-511CC were present in individuals with good response to splenectomy (p =0.05, OR = inf, CI = 0.94 to inf). **Conclusions.** With these findings it is proposed that the non carriers of allele 2 of the *IL1RN* VNTR polymorphism, confers risk for patients in the study as well as observed for *IL4* -590C/T polymorphism that could be a protective factor against development of disease. Also, patients with High Producer *IL10* haplotypes could have a greater chance of developing the disease. *IL1B*-511C/T polymorphism may be an important factor and that there is a tendency of those who are CC respond well to splenectomy.

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PLATELET SURFACE HIGH MOBILITY GROUP BOX PROTEIN-1 BINDS RECEPTOR FOR ADVANCED GLYCATION END-PRODUCTS IN PLASMA OR ON MONOCYTES IN SEPTIC PATIENTS

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Background. High mobility group box protein-1 is a cytokine mediator of inflammation in septic patients. Receptor for advanced glycation endproducts (RAGE) is a pattern recognition receptor, which has one of its ligands, HMGB1. Soluble form of RAGE levels increase several-fold in patients with sepsis. Since platelets play an important role in sepsis, it was hypothesised that platelet surface HMGB1 may bind to soluble RAGE. **Methods.** Flow cytometry (FACS) and Western blotting experiments were performed to identify RAGE on the surface of platelets in septic platelet-rich plasma and washed platelets. Soluble RAGE levels were analysed in septic plasma by ELISA and correlated with clinical scores including platelet count. Surface HMGB1 was measured on the septic platelets to identify the ligand for soluble RAGE on platelets. Different stimuli were used to activate platelets to identify the surface expression. Blocking experiments were done to see if platelet-surface HMGB1 was involved binding to RAGE on activated monocytes (U937 cell line). **Results.** RAGE was not expressed by platelets but was noted to be increased on platelets in septic platelet-rich plasma. Platelet-associated RAGE was the soluble form and not the receptor. Levels of soluble RAGE was increased in septic plasma and correlated with platelet counts. Surface HMGB1 was increased on septic platelets and binds to soluble RAGE in septic plasma. Blocking experiments suggested platelet HMGB1may be involved in binding to RAGE expressed by activated monocytes. **Conclusions**. Platelet-surface HMGB1 binds to soluble RAGE in the plasma of septic patients. This ligand may be important in platelet-monocyte aggregation in sepsis through HMGB1-RAGE interaction.

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MEAN PLATELET VOLUME CAN PREDICT CEREBROVASCULAR EVENTS IN PATIENTS WITH SICKLE CELL ANEMIA

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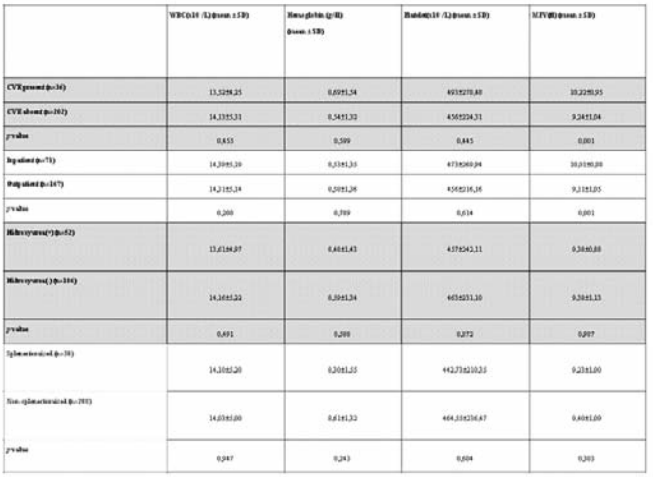
Background. - Sickle cell anemia (SCA) is characterized by intermittent vaso-obstructive events and chronic hemolytic anemia.**Aim** - The purpose of this study is to determine the impact of mean platelet volume (MPV) on the incidence and severity of vaso-occlusive and cerebrovascular events in patients with SCA. **Methods. -** The 238 cases diagnosed with SCA were evaluated retrospectively with respect to the occurrence of painful crisis for the previous year. The severities of vaso-occlusive crisis were determined by the need of hospitalization and site of vaso-occlusive crisis. **Results.** Of the SCA patients, 104 (43.7%) had 1-3 attacks (Group 1), 34 (14.3%) had 4-5 attacks (Group 2) and 100 (42%) had more than 6 attacks (Group 3). In accordance with the results obtained during the evaluation of the cases diagnosed with sickle-cell anemia, MPV value was found to be significantly higher in patients with cerebrovascular events. Also MPV values increased with increasing incidence of the crises (r=0.297) (p=0.001). **Conclusions.** One of the contributing factors for this clinical heterogeneity may be related to the MPV values in patients with sickle cell anemia. The higher MPV values may be an early predictor of future cerebrovascular events in patients with sickle cell anemia and may require close follow-up and additional measures.

Table 1: Some characteristics of patients in relation with the hematological parameters (CVE: Cerebrovascular event; MPV: mean platelet volume)



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ROMIPLOSTIM ALLOWS CHEMOTHERAPY ADMINISTRATION IN A PATIENT WITH HEPATOPATHY-ASSOCIATED THROMBOCYTOPENIA

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Background. Thrombocytopenia is a frequent complication of chronic liver disease and is considered an indicator of advanced disease. Among other factors, this low platelet count is due partly to decreased thrombopoietin production. Romiplostim is a protein that acts as a thrombopoietin receptor agonist, thus increasing platelet counts. Romiplostim and other orally administrated drug, eltrombopag have been approved by FDA and EMEA for use in immune thrombocytopenia. Use of thrombopoietin receptor agonists in liver disease is troublesome because of the risk of portal vein thrombosis, but has been safely administrated in cirrhosis associated with hepatitis C.Case Report: We report the case of a 63-year-old female with alcoholic cirrhosis in a Child-Pugh stage A6, abstinent for the last 4 years. She had been diagnosed of ductal carcinoma of breast 12 years before and remained in remission after surgery and tamoxifen therapy. When relapse occurred, it was found to be positive for estrogen receptor and c-erbB-2. Therapy with trastuzumab and paclitaxel was planned, but thrombocytopenia precluded paclitaxel use since platelet counts were 50,000/µL. Then romiplostim was started in a named patient basis in order to increase platelet counts. Eltrombopag has extensive experience in hepatopathy associated thrombocytopenia, but was discarded because of pharmacologic interaction with paclitaxel. On the other hand, according to product information, romiplostim is allowed for use in patients with Child-Pugh score below 7, but eltrombopag cannot be used with scores over 5.Dose schedule of romiplostim was the same as in immune thrombocytopenia, starting with 1 µg/kg and increasing 1µg/kg per week as to reach a target of 100,000/µL (higher than in immune thrombocytopenia). This was accomplished at 6 µg/kg and allowed paclitaxel therapy to be administered as scheduled except for a febrile neutropenia episode that delayed one dose. Platelet counts were maintained between 100,000 and 150,000/µL throughout the whole course of chemotherapy without any change in dose requirements.As well as physical examination, D-dimer was determined with every platelet count in order to screen for thrombosis. Moreover, a Doppler ultrasound scan has been performed every other month in order to rule out asymptomatic portal vein thrombosis. Despite increasing D dimer measurements, no thrombotic complications have been detected. **Conclusions.** Thrombopoietin receptor agonists can make possible chemotherapy administration when low platelet counts are the main limitation, although in an off-label use. Agent selection must carefully consider comorbidities, side effects and drug interactions. Surveillance of side effects must also be tailored to the individual patient

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ACTIVATION AND FUNCTION OF ELTROMBOPAG-INDUCED PLATELETS COMPARED TO PLATELET FUNCTION IN CORTICOID- TREATED AND UNTREATED ITP PATIENTS

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Background. Eltrombopag is approved for treatment of chronic immune thrombocytopenia (ITP). So far, no systematical analysis of platelet function in eltrombopag-treated ITP patients in comparison to steroid-treated or untreated ITP patients has been performed.**Aims.** We aimed to assess a possible influence of eltrombopag on platelet function *in vivo*. *Patients/* **Methods.** Platelet function in chronic ITP patients after eltrombopag-induced platelet rise (group 1; n=10) was compared to a control group (group 2; n=12) of patients on continuous therapy with corticoids and untreated ITP patients in a non-randomized prospective single-center study. All patients gave written informed consent.Platelet function was assessed both, naive and after in vitro addition of agonists at 2 different time points and averaged. Platelet adhesion under high shear conditions (surface coverage, SC), P-selectin expression, and formation of platelet-monocyte aggregates (PMA) served as parameters of platelet function.Data are given as median [1st-3rd quartile]. Group 1 is listed first. In case of normally distributed data, the Student´s t- tests, and in case of non-normally distributed parameters the nonparametric Mann-Whitney U test were used to compare the groups.*Results.* Eleven patients (female=9) were included in the treatment group with eltrombopag (group 1). The control group (group 2) consisted of 6 (female=4) patients on ongoing steroid treatment and 6 untreated (female=5) patients. One eltrombopag-treated patient was not included in the final comparative platelet function analysis, because he had no treatment induced platelet rise. None of the included patients developed severe bleeding during the study period, and none received rescue medication. Two patients developed venous thrombosis during eltrombopag treatment.Platelet counts [10^9/L] were 49.5 [45.00-59.00] in group 1 after eltrombopag-induced platelet rise, and 70.0 [64.0-80.0] in the control group. Naïve SC showed no difference between eltrombopag-treated patients and the control group with median levels of 5.80 [1.80-9.00] and 6.23 [5.03-11.75], respectively (p=0.488). Neither did activation with ADP (1.55 [1.05-2.70] and 1.80 [1.25-2.35]; p=0.692) or collagen (2.15 [1.90-2.30] and 2.75 [1.30-3.70]; p=0.339) reveal any difference in platelet activatability. Still, SC after addition of TRAP was decreased in the eltrombopag treatment group (1.00 [0.60-2.95] and 2.63 [1.90-4.48]; p=0.032).There were no differences in P-selectin expression [GeoMFI], neither without nor with addition of ADP, TRAP or collagen. The formation of PMA with and without agonistic stimulation was comparable in both groups. Median levels after addition of ADP were 18.01 [10.15-53.13] and 27.23 [15.64-34.05] (p=0.489), and 47.24 [32.24-58.57] and 29.18 [21.84-44.09] (p=0.070) after addition of TRAP.*Conclusion:* We proofed a good functional competence of eltrombopag-induced platelets. No substantial hyperreactivity or increased activatability of eltrombopag-induced platelets were identified in comparison to platelets of untreated or steroid-treated ITP patients.(Funded by GlaxoSmithKline, ClinicalTrials.gov number NCT00888901).

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WAIT AND SEE IS IT THE BEST THERAPY FOR IMMUNE THROMBOCYTOPENIC PURPURA IN CHILDREN?

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Background. Immune thrombocytopenic purpura (ITP) occurs in acute form in 75%-85% of affected children, with complete recovery within 6 months with or without therapy. Optimal therapy for ITP remains unknown. The need of treatment is based on the low but definitive risk of intracranial hemorrhage. The opinions about the need and type of therapy are contradictory. **Aims.** The aim of the present study was to assess the efficacy of different methods oftreatment ITP in children. **Methods.** During the 1980-2010 period, 74 male and 68 female aged 0-16 years with ITP were treated withcorticosteroids or IVIG or their combination, or were only observed. The use of therapy was primarily indicated by clinical picture rather than platelet count. Of 142 patients,101(71%) received no therapy, whereas 41(29%) patients with pronounced hemorrhagic diathesis and very low platelet count administered therapy as follows: steroids in 24(16.9%), IVIG in 14(9.8%), and a combination of steroids and IVIG in three (2.1% patients) for intracranial hemorrhage in two patients, and for emergency operativeprocedure for appendicitis in one patient).Results. All patients are alive to the present. Platelet count normalized within 6 months in 98(69%)patients, i.e.78 (54.9%) without therapy and 28(19.7%) with therapy, in 9(6.3%) within 5 years. Recurrent ITP developed in 11 (7.7%) and chronic ITP in the remaining 24(16.9%) patients.Conclusions: Accordingly, we believe that most children with ITP require no therapy.

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PLATELET MORPHOLOGY IN PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM: RESULTS FROM A CROSS-SECTIONAL STUDY

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Background. Subclinical hypothyroidism (SH) is often seen in the general population. Patients may be asymptomatic or may present non-specific symptoms. They are often diagnosed via routine screening and they have normal serum values of thyroxine (T4), triiodothyronine (T3) and mildly elevated serum concentrations of thyroid stimulating hormone (TSH). On the other hand, platelet parameters and especially mean platelet volume, an important determinant of platelet function, morphology and activation, constitutes a novel emerging risk factor for atherosclerosis and its complications such as coronary heart disease. Increased mean platelet volume (MPV) reflects active and large platelets that could release more thromboxane than smaller ones. It has been suggested that SH could represent a risk factor for cardiovascular disease, especially coronary heart disease. MPV, platelet distribution width (PDW), platelet count (PLT) have not been studied in depth in subclinical hypothyroidism.**Aims.** The aim of the present study was to compare the platelet count as well as the platelet indices MPV and PDW in SH and in euthyroidic healthy subjects and to investigate whether SH may possess a predictive significance in the determination of platelet morphology and function. **Methods.** In a cross-sectional study between 2009 and 2011, we have evaluated forty three patients with SH prior to any therapeutic intervention with levothyroxine (33 women and 10 men) with a mean age: 34.8 ± 8.3 years (range: 18-50 years) and an equal number of euthyroidic healthy subjects (33 women and 10 men) with a mean age: 33.9 ± 8 years (range: 20-48 years). Healthy subjects were matched on gender, age (±5 years), body mass index (± 1.5 kg/m^2) and year/month of diagnosis (±1 month). None of the subjects (patients and controls) presented any infectious and neoplastic conditions, diabetes mellitus, hypertension and dyslipidemia. To assess thrombopoiesis, we have determined platelet indices using the Sysmex 9000 analyzer. TSH, T3, free-T3, T4 and free-T4 were determined using an electro-chemiluminescence immunoassay intended for use on Elecsys 2010 analyzer (Roche Diagnostics). Statistical analysis of data was performed with IBM-SPSS® statistical package version 20 for Windowssoftware.Results. Cases presented significantly higher MPV (mean ± SD: 12.31 fL ± 0.45) and PDW (mean ± SD: 15.3 % ± 1.1) than controls (mean MPV ± SD: 10.7 fL ± 0.82, p<0.001 and mean PDW ± SD: 13.4 % ± 1.42, p<0.001). On the contrary, patients with SH exhibited similar number of platelets per mm^3 than healthy euthyroidic controls (mean PLT in patients: 250 x 10^3/mm^3 ± 37 versus mean PLT in controls 262 x 10^3/mm^3 ± 37, p=0.24). In a linear regression model, adjusting for age, gender, body mass index and smoking status, the diagnosis of SH was the most significant predictor of MPV and PDW levels (p<0.001). **Conclusions.** These results may suggest that subjects with SH tend to present an augmented platelet size. Elevated platelet activation could contribute to an increased risk of cardiovascular complications observed in SH. Finally, these findings suggest that platelet morphologic changes observed in SH, such as higher MPV and PDW, could be attributed to metabolic parameters.

Targeted therapies

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MLN4924, AN INVESTIGATIONAL NEDD8-ACTIVATING ENZYME (NAE) INHIBITOR, IN PATIENTS WITH RELAPSED AND/OR REFRACTORY LYMPHOMA OR MULTIPLE MYELOMA (MM): PHASE 1 DOSE-ESCALATION STUDY OF TWICE-WEEKLY DOSING

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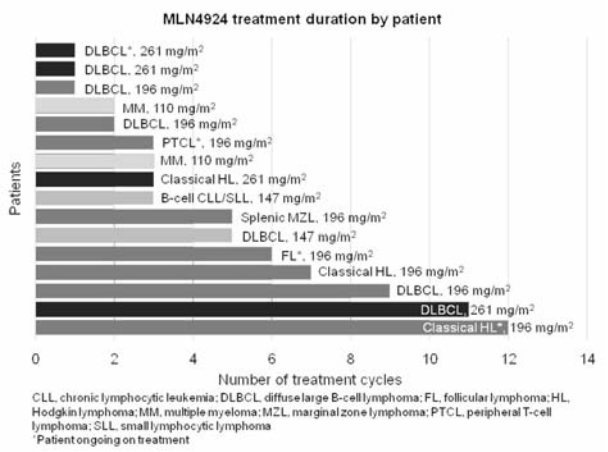
Background. MLN4924 is an investigational, first-in-class, small-molecule NAE inhibitor that prevents neddylation of cullin-RING ligases (CRLs) and consequently inhibits proteasomal degradation of CRL substrate proteins. MLN4924 induced apoptosis in lymphoma cell line studies and, *in vivo,* resulted in tumor growth inhibition/regressions in lymphoma xenograft models. Using a dosing schedule of days 1, 2, 8, and 9, 21-day cycles, the MLN4924 maximum tolerated dose (MTD) in patients with relapsed and/or refractory lymphoma or MM was 110 mg/m^2 (Shah *et al*, EHA 2010; dose-limiting toxicities [DLTs]: muscle cramps, febrile neutropenia, AST elevation, myalgia). Here we report an alternative twice-weekly MLN4924 dosing schedule. **Aims.** Objectives included: determining the MTD and safety of MLN4924; describing pharmacokinetics and pharmacodynamics; and evaluating response. **Methods.** Patients aged ≥18 years with relapsed and/or refractory B-/T-cell non-Hodgkin's lymphoma, Hodgkin lymphoma (HL), or MM following ≥2 prior lines of therapy received MLN4924 via 60-minute intravenous infusion on days 1, 4, 8, and 11 of 21-day cycles. Dose escalation proceeded from 110 mg/m^2 in 1.33-fold increments using a Bayesian continual reassessment method based on cycle 1 DLTs. For MLN4924 plasma pharmacokinetics analysis, serial blood samples were obtained during cycle 1. For pharmacodynamic analyses, peripheral blood mononuclear cells (PBMCs) and whole blood were isolated at baseline and following MLN4924 administration; skin biopsies were performed at baseline and after the second dose.



Results. Sixteen patients (12 male) were enrolled, including 2, 2, 8, and 4 to dose levels of 110, 147, 196, and 261 mg/m^2, respectively. Median age was 59.5 years (range, 26-68). Median time since primary diagnosis was 35.5 months (range, 2-101). Primary diagnoses were diffuse large B-cell lymphoma (DLBCL; n=7), classical HL (n=3), B-cell chronic lymphocytic leukemia, follicular lymphoma (FL), splenic marginal-zone lymphoma, peripheral T-cell lymphoma (PTCL; each n=1), and MM (n=2). One DLT was reported: grade 3 MLN4924-related thrombocytopenia (110 mg/m^2). Based on observed toxicities in all enrolled patients, the MTD was determined as 196 mg/m^2 using this twice-weekly schedule. Patients received a median of 3 treatment cycles (range, 1-12); at data cut-off (January 24), 4 were ongoing (Figure). The most common adverse events were fatigue (75%), nausea (69%), vomiting (63%), and decreased appetite (56%). Twelve (75%) patients experienced grade ≥3

events; only neutropenia and pneumonia (each n=2) were reported in >1 patient. In 11 evaluable patients, MLN4924 plasma exposure did not accumulate following multiple dosing, but maximum observed concentration increased more than dose-proportionally over the range studied. MLN4924 induced increased levels of Nrf-2-regulated gene transcripts/pIκBα in PBMCs and Cdt-1/Nrf-2 in skin biopsies. Two patients (DLBCL, PTCL; 196 mg/m^2) had a partial response, and 11 patients had stable disease, with disease control for up to 11 cycles. **Conclusions.** The MLN4924 MTD was 196 mg/m^2 using the twice-weekly schedule studied. Only neutropenia and pneumonia were reported as grade ≥3 toxicity in >1 patient. MLN4924 resulted in increased levels of the CRL substrates pIκBα, Cdt-1, and Nrf-2, the expected pharmacodynamic effects of NAE inhibition. Clinically meaningful responses (DLBCL, PTCL) and durable disease control (FL, HL, DLBCL) were seen in heavily pretreated patients.

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SMALL MOLECULES TARGETING ROR1 INDUCE SPECIFIC KILLING OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS

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Background. There is a great interest to develop targeting drugs for CLL to improve the outcome for the disease. ROR1 is a receptor tyrosine kinase, overexpressed on CLL cells but not on normal cells. ROR1 is constitutively phosphorylated and siRNA transfection induces leukemic cell death. **Aims.** Produce small molecules inhibiting the cytoplasmic tyrosine kinase activity of ROR1 and subsequent specific killing of leukemic cells. **Methods.** A high-throughput screening assay in 384-format has been developed measuring phosphorylation of a substrate peptide by a human recombinant intracellular ROR1 kinase domain. A collection of 80.000 small molecules has been screened generating two chemical series. Novel leads have been synthesized and structure-activity-relationship has been developed using the assay. **Results.** Five compounds were selected (KAN0173631, KAN0438063, KAN0438175T, KAN0438427T, KAN0438434). Freshly isolated leukemic CLL cells were used as targets as well as PBMC from healthy donors. The five compounds induced specific apoptosis of CLL cells (Annexin V/PI and MTT)(24h). Efficacy index (EI), i.e. killing of leukemic cells in relation to normal PBMC was favourable. The most promising drug KAN0438063 (IC_{50} 10μM) had an EI of 40 i.e. killed 40 times more CLL cells than PBMC at a conc. of 10μM. The compounds induced down regulation of PARP and caspases 8 and 9 as well as down regulation of Mcl-1 and Bcl-xl. ROR1 was dephosphorylated as PKC and ERK (Western Blot). The selective apoptotic effect was compared to other small molecules targeting non-ROR1 structures in CLL (PCI-32765, CAL-101, R406, R788, STK-156485, STK-156133) and our compounds were significantly more effective (EI)($p<0.001$). **Conclusions:** We have developed effective and selective series of compounds targeting ROR1 with promising ADMET properties. ROR1 targeting small molecules might also be effective for other tumor cells expressing ROR1 as other lymphoid and myeloid malignancies. Our model molecules will be further optimized and tested in animal tumor models. These molecules represent the first small molecules targeting ROR1 - a “survival factor”in CLL.

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GS-1101 (CAL-101), A SELECTIVE PHOSPHATIDYLINOSITOL 3-KINASE-DELTA INHIBITOR, IN COMBINATION WITH OFATUMUMAB FOR THE TREATMENT OF RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. PI3Kδ is predominantly expressed in cells of hematopoietic origin where it regulates the survival and proliferation of malignant B-cells. GS-1101 is an orally bioavailable, small-molecule inhibitor that selectively targets PI3Kδ and is highly active in patients with hematologic B-cell malignancies. **Methods. and Patients.** This Phase 1/2 study evaluated repeated 28-day cycles of GS-1101 (CAL-101) in combination with ofatumumab for the treatment of relapsed or refractory chronic lymphocytic leukemia (CLL). GS-1101 (150mg BID) was co-administered with a total of 12 infusions of ofatumumab over 24 weeks (300mg initial dose either on Day 1 or Day 2 (relative to the first dose of GS-1101), followed 1 week later by 1000mg weekly for 7 doses, followed 4 weeks later by 1000mg every 4 weeks for 4 doses). Start of ofatumumab treatment either on Day 1 or on Day 2 allows for evaluating an additional beneficial effect of GS-1101 on ofatumumab-related infusion reactions. After finalizing the ofatumumab treatment, each subjects received single-agent GS-1101 as long as the subject was benefiting. **Results.** At the time of abstract submission, accrual was complete with 21 subjects enrolled and 11 evaluable. Median [range] age was 63 [54-76] years. The majority (9/11; 82%) of patients had bulky adenopathy. The median [range] number of prior therapies was 3 [1-6], including prior exposure to alkylating agents (10/11; 90%), rituximab (9/11; 82%), purine analogs (8/11; 72%), alemtuzumab (3/11; 28%) and/or ofatumumab (2/11;18%). At the data cutoff, the median [range] treatment duration was 5 [0-7] cycles. Almost all subjects (9/11;82%) experienced marked and rapid reductions in lymphadenopathy within the first 2 cycles**.** The lymphocyte mobilization that is expected with PI3Kδ inhibition was significantly reduced in magnitude and duration and persisted past Cycle 1 in only 1 patient. Early follow up data support a favorable safety profile and confirm a lack of clinically significant myelosuppression. Elevated baseline levels of CCL3, CCL4, CXCL13, and TNFα were significantly reduced after 28 days of treatment.**Conclusions.** The combination of GS-1101 (CAL-101) and ofatumumab offers a well-tolerated non-cytotoxic regimen with substantial activity in patients with previously treated CLL. Updated data on the complete cohort of 21 subjects will be presented.

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ONCE-WEEKLY MLN9708, AN INVESTIGATIONAL PROTEASOME INHIBITOR, IN PATIENTS WITH RELAPSED/REFRACTORY LYMPHOMA: RESULTS OF A PHASE 1 DOSE-ESCALATION STUDY

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Background. MLN9708 is a reversible, orally bioavailable, specific 20S proteasome inhibitor. This is the first study of IV MLN9708 in patients with relapsed/refractory lymphoma (NCT00893464).**Aims.** To determine the safety, maximum tolerated dose (MTD), pharmacokinetics (PK), and pharmacodynamics (PD) of once-weekly IV MLN9708 in patients with relapsed/refractory lymphoma. **Methods.** Patients aged ≥18 years with confirmed lymphoma who had failed ≥2 chemotherapeutic regimens received IV MLN9708 on days 1, 8, and 15 of 28-day cycles until disease progression or unacceptable toxicity. One patient was enrolled at a starting dose of 0.125 mg/m^2; dose doubling proceeded with 1 patient per dose up to 1.0 mg/m^2. Dose escalation occurred in 30-40% increments using a standard 3+3 scheme based on occurrence of dose-limiting toxicites (DLTs) in cycle 1. Toxicity was evaluated using NCI CTCAE v3.0. Blood samples were collected at multiple time points after dosing on days 1 and 15 of cycle 1 for PK/PD analyses. Response was assessed using IWG criteria. **Results.** At data cut-off (Feb 10, 2012), 22 patients had been enrolled: 1 each at 0.125 and 0.25 mg/m^2, 2 at 0.5 mg/m^2, 1 at 1 mg/m^2, 4 at 1.4 mg/m^2, 7 at 1.76 mg/m^2, and 6 at 2.34 mg/m^2. Twenty-one patients were included in the safety population. Median age was 57 years (range 23-78); 57% were male. Median number of prior therapies was 5; 32% had received prior radiation and 23% prior stem cell transplant. Median time from last dose of prior therapy to first dose of MLN9708 was 5 months (range 0.6-17.2). Histologies included follicular lymphoma (FL; n=7), T-cell lymphoma (TCL; n=5), DLBCL (n=4), Hodgkin lymphoma (n=3), and other (n=2). Patients received a median of 2 cycles (range 1-22). Two DLTs were seen (grade 4 neutropenia at 1.76 and grade 3 neutropenia at 2.34 mg/m^2); MTD has not yet been reached. All patients experienced adverse events (AEs); the most common all-cause AEs included fatigue (n=13), cough, diarrhea, headache, nausea, and pyrexia (each n=7). The most common drug-related AEs included fatigue (n=10), nausea (n=6), diarrhea (n=6), pyrexia, thrombocytopenia, and vomiting (each n=5). Nine patients had drug-related grade ≥3 AEs. One patient receiving 2.34 mg/m^2 discontinued due to drug-related grade 3 neutropenia. Three patients had drug-related peripheral neuropathy (1 grade 1, 2 grade 2). One on-study death occurred, due to respiratory failure unrelated to MLN9708. Of 20 response-evaluable patients, 3 achieved partial response (including 2 with FL and 1 with TCL) and 4 had stable disease. The 2 FL patients remain in response: 1 achieved PR after cycle 8 (now in cycle 23) and the other achieved PR after cycle 4 (now in cycle 16). PK analyses showed linear PK (0.5-2.34 mg/m^2) and a terminal half-life of ~6-9 days. There was a dose-dependent increase in maximal whole blood 20S proteasome inhibition.**Conclusions:**These phase 1 data suggest that once-weekly IV MLN9708 is generally well tolerated, with infrequent PN, and signs of clinical activity in heavily pretreated lymphoma patients. The trial is ongoing and updated data will be presented.

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THE IMPACT OF ENTERAL GRANULOCYTE COLONY STIMULATING FACTOR AND ERYTHROPOIETIN ON FEEDING TOLERANCE IN PRETERM INFANTS

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Background. Feeding intolerance in premature neonates and prolonged times of nothing per os (NPO) result in intestinal villous atrophy and subsequent increase in the incidence of necrotizing enterocolitis (NEC). Intestinal growth factors could help prevent villous atrophy. **Aims.** This study aimed at evaluating the benefit of granulocyte colony stimulating factor (G-CSF) and erythropoietin (EPO) in preventing feeding intolerance when given enterally to achieve maximum effects on neonatal bowel and to minimize systemic side effects. **Methods.** The present study is a double-blind randomized case-control study carried out on 90 preterm infants less than 33 weeks gestational age. The study protocol was reviewed and approved by the Research Ethical Committee of Ain Shams University under ID number FMASU 548/2010. This trial is registered at ClinicalTrials.gov; identifier: NCT01441427. The infants who met study criteria were identified and informed consents were obtained from parents. Newborns with congenital anomalies of gastrointestinal tract or major congenital anomalies were excluded. Neonates were assigned to 4 groups; 20 received rhG-CSF, 20 received rhEPO, 20 received both and 30 received plain distilled water (control). Treatment was started with the start of enteral feeding. The test solution (or placebo) was discontinued if enteral intake >80 ml/kg of milk any time even before 7 complete days of treatment. Feeding tolerance and adverse effects of treatment (if any) were assessed by clinical follow up. **Results.** All the studied neonates tolerated the received treatment well without side effects that could be attributed to drug intake. The study group showed better feeding tolerance as reflected by earlier achievement of full feeds, earlier weight gain and lesser hospital stays. The incidence of NEC was significantly decreased among treatment groups. The best feeding tolerance was found among neonates receiving both G-CSF and EPO. No significant difference was found between serum G-CSF or EPO levels in days 0 and 7 among the three treatment groups. **Conclusions:** This study provides further insights on the improvement of neonatal outcomes and help decrease morbidities from malnutrition and feeding intolerance in premature babies. Enteral administration of rhG-CSF and rhEPO may play a critical role in preventing villous atrophy, thereby, reducing feeding intolerance and NEC. Serum levels of the studied intestinal growth factors were stable with no difference before initiation and after cessation of treatment. Larger prospective multicenter studies are required to further assess the safety and side effects of these factors before their clinical application on a wider scale.

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PRE-CLINICAL ACTIVITY OF A NOVEL CRM1 INHIBITOR IN ACUTE MYELOID LEUKEMIA

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Background. CRM1 is a nuclear export receptor involved in the active transport of numerous cargo proteins, including transcription factors, tumor suppressors and cell-cycle regulators such as p53, p21/27, and nucleophosmin (NPM1). The mislocalization of a nuclear protein in the cytoplasm can render it ineffective as a tumor suppressor. Therefore, blocking CRM1 mediated export of proteins using CRM1 inhibitors is a novel and attractive anti-neoplastic therapeutic modality. Karyopharm Therapeutics has developed small molecule Selective Inhibitors of Nuclear Export (KPT-SINEs) that specifically and irreversibly bind to CRM1 and block the function of this protein. **Aims.** To investigate the anti-leukemic activity of KPT-185 and KPT-276 in AML cell lines and patient samples and to establish in-vivo efficacy using a murine xenograft AML model. **Methods.** Cell proliferation (MTS assay), apoptosis (Annexin V), cell-cycle (PI) and differentiation (FACS and Morphology) were assessed in AML cell lines and primary samples after KPT administration. To investigate the in-vivo anti-leukemic activity of the KPT-SINEs, we use an established xenograft mouse model of AML, where human MV4;11 leukemic cells were injected into NOD/SCID/IL-2Rgamma deficient mice. **Results.** KPT-185 displays potent anti-proliferative properties in AML cell lines (MV4;11, Kasumi-1, OCI-AML3, THP-1, KG1a, MOLM-13) as well as in 6 primary AML samples (including CN-AML with NPM1 mutated, FLT3-ITD+, t(8;21) and inv16). The IC50 values ranged from 100nM to 500nM. KPT-185 induces cell-cycle arrest in cell lines at G1 (82.64±0.42% vs. 61.4±8.87%, p=0.008) with a concomitant decrease in the percentage of cells in S (5.34±1.07% vs. 21.51±5.08%, p=0.003) and G2/M phase (4.55±0.25% vs. 12.46±3.33%, p=0.008) at 24 hrs. Apoptosis was induced (average 50%) by KPT-185 in all cell lines and primary AML samples compared with controls (DMSO). Remarkably, granulocytic differentiation was observed in MV4;11 and Kasumi-1 cells after KPT-185 treatment as measured by CD11b (CD11b MFI, DMSO vs. KPT, 264±1.41 vs. 513±16.97, p=0.002 and 617.66±40.77 vs. 2084.5±396.68, p=0.003, respectively) and morphology. Treatment with KPT-185 in AML cell lines results in significant nuclear accumulation of CRM1 cargo proteins such as p53 and NPM1. Interestingly, we also found strong downregulation of FLT3 and c-KIT protein expression, while CEBPA protein and mRNA expression were up-regulated. Due to the critical role of CEBPA in myeloid differentiation and our findings that KPT-185 induces blast differentiation, we further investigated the mechanisms by which KPT-185 up-regulates CEBPA. We found that CEBPA upregulation is mediated through p53, since blocking p53 using siRNA in MV4;11 and OCI-AML3 abrogates CEBPA upregulation induced by KPT-185. Finally, using our xenograft tumor mouse model, we show that in vivo treatment of mice with oral KPT-276 (ana-

log of KPT-185 for in vivo studies) significantly prolongs survival of leukemic mice (median survival, vehicle vs. drug treated, 27 vs. 39.5 days, p=0.0002, log-rank test, n=12 per group). **Conclusions.** KPT-SINE CRM1 inhibitors are highly potent in AML and prolong survival in a mouse model of AML. Mechanistically, we show that blast differentiation after KPT-SINE treatment is mediated through P53-CEBPA and identified FLT3 as a novel pharmacodynamic endpoint for testing in Phase 1 clinical trials.

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SYNERGISTIC COMBINATION OF MLN4924, AN INVESTIGATIONAL SMALL MOLECULE INHIBITOR OF NEDD8-ACTIVATING ENZYME (NAE), WITH AZACITIDINE, A HYPOMETHYLATING AGENT, IN PRE-CLINICAL AML CANCER MODELS

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Background. MLN4924 is an investigational small molecule inhibitor of NEDD8-activating enzyme (NAE). NAE regulates cullin-dependent ubiquitin E3 ligases (CDLs) which target a subset of cellular proteins for ubiquitination and degradation by the proteasome. MLN4924 treatment of cultured cells causes accumulation of these proteins and can cause DNA re-replication, DNA damage and apoptosis. MLN4924 has shown some limited signs of clinical activity in a Phase I trial of acute myelogenous leukemia (AML). **Aims.** To evaluate the effects of MLN4924 on cell cycle and viability in a variety of AML cell lines, as a single agent and in combination with other drugs, and to further characterize a synergistic combination in xenograft models of AML grown in immunocompromised mice. **Methods.** HL60, OCI-M2, THP-1 and NB4 cells were treated with MLN4924 in vitro. Cell cycle distribution was measured by flow cytometry. Apoptosis was measured by Western blot for cleaved caspase-3 and flow cytometry for annexin V. A high-throughput combination screen was performed with the Cell Titer Glo® viability assay in a 384 well format using AML cell lines treated with MLN4924 and approximately 40 compounds covering a wide variety of mechanisms of action. Synergy was determined by combination index. For in vivo combination studies, HL60, THP-1 and OCI-M2 xenograft-bearing mice were dosed subcutaneously with MLN4924 and azacitidine as single agents or in combination on Days 1, 4, 8, 11, 15 and 18.Results. In vitro characterization of AML cell lines revealed that all 4 lines were sensitive to MLN4924 with EC50 <500 nM, but there were two distinct cell cycle phenotypes following MLN4924 treatment. OCIM2 and THP1 demonstrated moderate S-phase accumulation with greater than 4N DNA content, consistent with DNA re-replication. In contrast, HL60 and NB4 demonstrated rapid accumulation of subG1 cells without S-phase accumulation or DNA re-replication, suggesting faster progression to apoptosis. The combination screen identified 2 hypomethylating agents (azacitidine and decitabine) as synergistic with MLN4924 in multiple AML cell lines. The combinations significantly increased apoptotic cell death compared to single agent alone. In vivo studies in the HL-60 xenograft model demonstrated marginal single-agent activity of MLN4924 and azacitidine, whereas co-dosing of MLN4924 and azacitidine led to complete and sustained tumor regressions in a subcutaneous xenograft model and significantly prolonged survival in a disseminated model. In the THP-1 xenograft model, single agent azacitidine showed limited anti-tumor activity whereas MLN4924 inhibited tumor growth, but only co-administration of both MLN4924 and azacitidine led to tumor regressions. Additionally, the combination treatment group showed a statistically significant delay in tumor regrowth after the end of the treatment period compared to the MLN4924 group. In the OCI-M2 xenograft model, MLN4924 and azacitidine as single agents significantly inhibited tumor growth, while the combination produced complete tumor regressions. The mechanism of in vivo synergy is currently under evaluation; initial studies have shown a dramatic elevation in cleaved-caspase 3 in the tumors treated with the combination as compared to the single agent. Thus MLN4924 and azacitidine can combine to produce synergistic antitumor activity in pre-clinical models of AML.

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PONATINIB AS TARGETED THERAPY FOR THE 8P11 MYELOPROLIFERATIVE SYNDROME

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The 8p11 myeloproliferative syndrome (EMS) is a rare, poor prognosis stem cell disorder that typically presents as an MPN with eosinophila and T- or B-cell lymphoma. EMS is characterised by chromosome rearrangements involving 8p11-12 that fuse several different partner genes to *FGFR1*, resulting in constitutively active tyrosine kinase fusion proteins. Although a number of FGFR1 inhibitors have shown in vitro activity against these disorders, none are in use clinically and there is a need to assess additional compounds as potential agents for the treatment of EMS patients. Ponatinib (AP24534) is a small molecule tyrosine kinase inhibitor with activity against ABL, SRC, FGFR, VEGFR and PDGFR family kinases currently in trials for chronic myeloid leukemia patients with resistance to currently available TKIs. We have examined its activity in in-vitro assays using cell line models and primary patient material from patients with *FGFR1* translocations. Activity was assessed in the KG1A cell line, which carries an *FGFR1OP2-FGFR1* fusion gene, and Ba/F3 cells transformed to IL3 independence by *BCR-FGFR1* (Ba/F3-BF) or *ZNF198-FGFR1* (Ba/F3-ZF). Non-transformed Ba/F3 cells and HL60 were used as negative controls. Cells were grown in a range of ponatinib concentrations for two days with proliferation and survival assessed by MTS assay. Ba/F3-BF, Ba/F3-ZF and KG1A showed reduced proliferation and survival (IC_{50} = 22 nM, 29 nM and 55 nM respectively) compared with untransformed Ba/F3 cells and HL60 (IC_{50} =8,690 nM and 585 nM respectively). Western blotting showed a dose-dependent reduction in phosphorylation of the FGFR1 fusion protein, STAT5 and ERK. Primary cells from patients with *FGFR1* fusion genes and from healthy controls were grown in methylcellulose for two weeks and colonies counted on days 7 and 14. An index of response was measured as the mean relative colony number at all inhibitor concentrations compared to untreated cultures. Median index values for patients (N=5) and controls (N=8) were 0.29 and 0.71 respectively indicating a greater reduction in colony growth in FGFR1 translocation patients compared with controls. Overall, reduced numbers of colonies were seen in patient cultures compared to controls at all ponatinib concentrations used. Fluorescence in situ hybridization on plucked colonies from one patient demonstrated a reduction in the proportion of *FGFR1* fusion gene positive colonies in ponatinib-treated versus untreated cultures. In summary we have shown that ponatinib has in-vitro activity against cells with *FGFR1* fusion genes and therefore shows promise for the treatment of patients with EMS.

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AKT INHIBITION - A NOVEL THERAPEUTIC AGENT IN REFRACTORY LANGERHANS CELL HISTIOCYTOSIS

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Background. Langerhans cell histiocytosis (LCH) is a rare clonal disorder of Langerhans cells with a broad disease spectrum. Trialled therapies include steroids, chemotherapy, radiotherapy and stem cell transplantation, but for patients with disease refractory to these modalities there are few options available.AKT (a serine/threonine) is a protein kinase involved in oncogenesis with key roles in cellular survival and proliferation. AKT inhibition is a novel therapeutic approach under evaluation in a variety of haematological malignancies. We present the case of an adult with heavily pretreated, refractory mediastinal and pulmonary LCH who achieved a sustained response after treatment with an oral AKT-inhibitor (GSK2110183) as part of a phase I, open-label study.**Case.** A 44 year old woman presented with cough, weight loss and sweats. CT & whole-body PET imaging revealed a large pulmonary and hilar mass accompanied by extensive mediastinal lymphadenopathy. Tissue biopsy confirmed LCH. Six cycles of conventional therapy consisting of 2-chlorodeoxyadenosine (0.14mg/kg/day for 5 days) and subsequent adjuvant local radiotherapy resulted in a partial response based on PET criteria. Within 12 months there was disease progression with additional mediastinal lymphadenopathy and pulmonary involvement confirmed by rebiopsy. Treatment with multi-agent conventional chemotherapy ("Third International Study for Langerhans Cell Histiocytosis protocol") for 6 months resulted in a partial PET response, but within 6 months, disease progression was evident with dysphagia secondary to external compression of the gastro-oesophageal junction. At this stage GSK2110183 was commenced (150mg daily, in 21-day cycles) with a rapid resolution of her dysphagia and cough after 2 cycles. CT and PET demonstrated reduction in size and activity of known lesions. No significant adverse events were experienced. She has now completed 25 cycles of therapy and remains asymptomatic with a sustained near complete response. **Results & Discussion.** Mutation analysis of the patient's cervical lymph node and mediastinal mass biopsy was performed by PCR using Sequenons OncoCarta™ Assay Panel v1.0. Dysregulation of the BRAF pathway has been suggested as a mechanism in the pathogenesis of LCH. The most common mutation, BRAF600VE causes constitutive activation of the BRAF protein and hence increased expression of its downstream molecules, pMEK and pERK. No mutations were found (including BRAF and AKT1, AKT2) in our patient's biopsy specimens. Immunohistochemistry revealed over-expression of phospho-AKT and moderate expression of pMEK, pERK and SK6. Cyclin D1, p53 and NFKB (all downstream molecules of AKT)

expression was low.Low expression of pMERK and pERK combined with absent BRAF mutations suggest the pathogenesis of LCH is due to an alternative pathway. Given the over-expression of phospho-AKT in out patient's sample and the response to AKT inhibition, we propose the AKT pathway is involved in the pathogenesis of LCH. **Conclusions.** The dramatic and sustained response to AKT inhibition in this case of refractory LCH provides a rationale for the re-evaluation of the pathogenesis of LCH and the mechanisms underlying the response.No conflicts of interest to disclose.

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SINGLE UK CENTRE EXPERIENCE OF BRENTUXIMAB VEDOTIN MONOTHERAPY IN REFRACTORY CD30+ LYMPHOMAS

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Background. The CD30-targeted agent brentuximab vedotin (BV) has shown impressive activity in relapsed/refractory Hodgkin lymphoma (HL) and Anaplastic Large Cell Lymphoma (ALCL) in two pivotal phase 2 studies. We present our single centre experience of BV in a population of patients with relapsed/refractory CD30+ lymphomas treated on a Named Patient Programme. **Aims**To describe objective response rates, adverse events, subsequent allotransplant rate, progression-free and overall survival. **Methods.** 24 patients with HL, ALCL or CD30+ T cell lymphoma refractory to at least 2 lines of chemotherapy or autotransplant, and fit for systemic therapy, were enrolled December 2010 - August 2011. BV was administered at a dose of 1.8mg/kg once every 3 weeks. Response was assessed by FDG-PET/CT using IWG criteria after cycles 4 and 8 (PET4, PET8) and additionally as clinically indicated. Eligibility for allotransplant was assessed per institutional guidelines (including age ≤65). Toxicity was graded by CTCAE v3.0.

Table 1. Results of the brentuximab vedotin NPP.

Median age (range)	41.5 (21,78) years
Female	13
Histology	18 HL, 5 ALCL, 1 CD30+ TCL
Median number of prior regimens (range)	3 (2,8)
Prior auto-HSCT	8/24 (33%)
Previous radiotherapy	10/24 (42%)
No response to most recent treatment	17/24 (71%)
Number eligible for allo-HSCT at baseline	22/24 (92%)
IWG Response after 4 cycles BV	ORR 67% (6 CR (25%), 10 PR (42%)). 4 PD
Best response to BV	6 CR, 10 PR
Median number of cycles of BV (range)	5.5 (1,13)
Disease status at end of BV	5 CR, 1 PR, 17 PD
Median PFS	156 days
Median OS	Not reached
Median Follow Up (from C1d1)	363 days
CTC AE > Grade 2 during BV treatment	9/24 (38%)
Proceeded to allo-HSCT	5/22 (23%) 3 CR (2 ALCL,1HL), 1 PR (HL), 1 PD*(HL)
Type of allo-HSCT	5 RIC (1 Sibling, 4 MUD)
Disease status after allo-HSCT	3 CR, 1 awaits assessment, 1 deceased
CTC AE > Grade 2 post allo-HSCT (inc. GvHD)	2 (Fatal CMV reactivation, colitis)

Results. Best responses were seen at PET4 with 6 CR, 10 PR and 4 PD at this time (Table). 4 patients experienced clinical progression or death before PET4. One PR patient discontinued therapy for personal reasons and has been excluded from PFS analysis.Most toxicity was mild to moderate: 9 patients experienced grade 3/4 events including sub-acute bowel obstruction (n=1), neuropathy (n=3) and sepsis (n=5, three of which fatal, including a case of EBV reactivation). 3 patients with neurotoxicity and 1 with neutropaenic sepsis at the time of CR were dose-reduced to 1.2mg/kg.At a median follow-up of 363 days, 16/24 patients are alive (67%) and median PFS in 23 patients is 156 days. 5/22 (23%) eligible patients (HL 3, ALCL 2) have undergone allotransplant and a further 2 await this procedure (HL 1, ALCL 1). One patient* in PD with a single new lymph node site following BV proceeded to allotransplant but subsequently died of CMV reactivation; all other patients were in CR (n=3) or PR (n=1) at allo-transplant. 12 patients potentially eligible for allotransplant at baseline did not proceed due to PD at PET4 (n=2) or PET8 (n=10). So far in early post-transplant follow-up, one patient converted from PR to CR and 1 patient has had treatment for severe colitis. There have been no other unexpected toxicity events. **Summary and Conclusions.**BV was well tolerated and effective in a population of heavily pretreated patients including a majority refractory to their most recent therapy. Response and survival rates were comparable to phase 2 data and 23% of our cohort (5/22 eligible on an intention-to-treat basis) proceeded to allogeneic transplantation following BV. The best response was observed at PET4 in all patients.For those deemed eligible for allotransplant, our data suggest that BV is an effective induction regimen in approximately 25% of patients with CD30+ lymphomas refractory to conventional salvage chemotherapy. Our observation that best response occurred at PET4 also supports the view that consideration of allogeneic transplantation should be made early in therapy and, if appropriate scheduled following the first assessment indicating response.

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RUXOLITINIB AS POTENTIAL TARGETED THERAPY FOR MYELOPROLIFERATIVE NEOPLASMS WITH JAK2 FUSION PROTEINS

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Chromosome translocations that target JAK2 are rare but recurrent abnormalities observed in myeloproliferative neoplasms (MPN), acute leukemia and lymphoma. Three fusion variants, *PCM1-JAK2*, *BCR-JAK2* and *ETV6-JAK2* have been described in MPN that give rise to constitutively active fusion proteins that are believed to drive the disease process in a manner analogous to *BCR-ABL* in chronic myeloid leukaemia. To date, however, no compound with activity against JAK2 has been investigated for this indication or approved for clinical use. Ruxolitinib (also known as INC424; Novartis/Incyte) is an orally available JAK1/2 inhibitor which has been approved by the US FDA for the treatment of intermediate or high-risk myelofibrosis and is being investigated for the treatment of other MPN. We have examined the activity of ruxolitinib against *JAK2* fusions using in vitro assays, cell line models and primary material from patients with *JAK2* rearrangements. Response was assessed in Ba/F3 cells transformed to IL3 independence by ectopic expression of *ETV6-JAK*. KG1A and Ba/F3 cells transformed by *BCR-ABL*, *ZNF198-FGFR1* or *SPTBN1-FLT3* were used as negative controls. Cell lines were grown in a range of ruxolitinib concentrations for two days and then assessed for proliferation and survival by MTS assay. *ETV6-JAK2* cell lines showed reduced proliferation and survival (mean IC_{50} = 697 nM) compared with all negative control cell lines (IC_{50} not reached at 10 μM for all). Western blotting demonstrated a dose-dependent reduction in phosphorylation of the JAK2 fusion protein, ERK and STAT5. Colonies from cryopreserved primary cells from two patients with MPN and *JAK2* rearrangements and from healthy controls were grown in methylcellulose containing 0, 20, 100 or 500 nM ruxolitinib and colonies were counted on day 7. An index of response was measured as the mean reduction in colony number compared to untreated cultures. Median index values for controls (N=7) and patients (N=2) were 0.48 and 0.23 respectively, i.e. a clear reduction in colonies grown from MPN patients with *JAK2*-rearrangements compared with controls ($p<0.05$). Overall, reduced numbers of colonies were seen in patient cultures compared to controls at all ruxolitinib concentrations used. Colonies were plucked into fixative for fluorescence in-situ hybridisation analysis using *JAK2* split-apart probes. Both patients showed a significant reduction in the proportion of *JAK2*-rearrangement positive colonies in treated compared to untreated cultures ($p<0.05$). One patient showed complete eradication of *JAK2*-rearrangement positive colonies at 500 nM ruxolitinib. Our in vitro assays therefore demonstrate that ruxolitinib has significant activity against cells containing *JAK2* rearrangements and shows promise for the treatment of patients with *JAK2* fusion gene positive MPN

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INDUCTION OF CELL DEATH BY APOPTOSIS SENSITIZING SMAC MIMETICS IN PRIMARY ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Acute lymphoblastic leukemia (ALL) is the most frequent malignant disease in childhood and adolescence. Although intensification of multiagent chemotherapy regimens have led to improvement of remission induction and long-term survival, patients with high risk ALL or relapse do not respond well to current treatments and still have a poor prognosis. Because this failure is, in part, due to defects in apoptosis programs, new strategies are required that counter apoptosis resistance in order to sensitize for cell death and improve prognosis. Since "Inhibitor of Apoptosis"(IAP) proteins are expressed at high levels in acute leukemia and block apoptosis at a central point of the apoptosis machin-

ery, they present a suitable molecular target for therapeutic intervention.We previously showed that the principle to neutralize IAP proteins by small molecule IAP inhibitors is an effective approach to sensitize childhood acute leukemia cells for death receptor- or chemotherapy-induced apoptosis. In this study we further extended our analyses and investigated the effect and mechanisms of new small molecules mimicking the IAP inhibiting molecule SMAC (second mitochondria derived activator of caspases), so called SMAC mimetics, on ALL cell lines and a panel of primary (patient or patient derived xenograft ALL).First, we used ALL cell lines and observed apoptosis induction upon SMAC mimetic treatment in a majority of cell lines at nanomolar concentrations. Interestingly, SMAC mimetic induced apoptosis by was dependent on a TNF-alpha feed forward loop in sensitive cell lines indicated by inhibited apoptosis induction by soluble TNF-alpha receptor (Etanercept).Moreover, the small molecule SMAC mimetic was tested on a variety of 30 primary ALL samples either directly obtained from patients at diagnosis (n=3) or isolated from ALL bearing mice of established patient derived NOD/SCID/huALL xenograft leukemia samples (n=27). Treatment with the SMAC mimetic at nanomolar concentrations showed a clear induction of cell death in the vast majority of primary ALL samples tested. In most of these SMAC mimetic induced cases cell death was inhibited by the soluble TNF-alpha receptor Etanercept indicating TNF-alpha dependency also in primary ALL.Intact apoptosome formation, a key downstream apoptogenic event, was functionally analyzed assessing mitochondrial cytochrome c release and activation of the effector caspase-3 in a subset of primary xenograft samples (Cytochrome c-related caspase-3 activation, CRAC). Interestingly, upon treatment with small molecule SMAC mimetics induction of cell death was also observed in primary xenograft ALL samples showing constitutive deficient apoptosome function. Furthermore, we previously described that rapid engraftment of ALL cells transplanted onto NOD/SCID mice analyzed as weeks from transplantation to onset of leukemia related morbidity in the recipients (short Time To Leukemia, TTL^{short}) is indicative for early patient relapse. Importantly, primary xenograft ALL samples with a TTL^{short}/early relapse phenotype showed increased cell death upon treatment with SMAC mimetic small molecules.Thus, induction of apoptosis by the new generation of small molecule SMAC mimetics provides a promising novel strategy for targeted therapy of high risk acute lymphoblastic leukemia.

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EFFECTS OF PONATINIB ON GROWTH AND SURVIVAL OF OF NEOPLASTIC HUMAN MAST CELLS

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Background. In patients with advanced systemic mastocytosis (SM) including aggressive SM (ASM) and mast cell leukemia (MCL), neoplastic cells usually harbor the D816V-mutated variant of KIT, that mediates resistance against several tyrosine kinase inhibitors (TKI). The response to conventional or targeted therapy in advanced SM is poor and the prognosis is grave. Recently, novel TKI, such as midostaurin (PKC412), have been described to block KIT D816V and to inhibit the proliferation of neoplastic MC. However, in clinical trials, midostaurin failed to induce long-lasting complete remissions in most patients with ASM or MCL. A possible reason for non-response/relapse may be the activation of KIT D816V-independent oncogenic pathways contributing to abnormal survival of neoplastic MC. Therefore, combinations of TKI covering a large spectrum of targets may represent an interesting approach in advanced SM. Ponatinib is a novel multikinase-inhibitor applied in TKI-resistant CML. **Aims.** We explored the effects of ponatinib as single agent or in combination with midostaurin on neoplastic MC. **Methods.** Primary neoplastic mast cells and the MCL cell line HMC-1 were used. Effects on kinase phosphorylation were evaluated by Western blotting. Cell proliferation was measured by ^{3}H-thymidine uptake. Apoptosis was determined by light microscopy, flow cytometry and by a TUNEL assay. **Results** Ponatinib was found to inhibit the phosphorylation of a number of pro-oncogenic molecules in HMC-1 cells, including KIT D816V, Lyn, and STAT5. In addition, ponatinib induced growth inhibition and apoptosis in HMC-1.1 cells (KIT G560V+) and HMC-1.2 cells (KIT G560V+ and D816V+). These effects were dose-dependent, with 100-fold higher IC_{50}-values in HMC-1 cells harbouring KIT D816V compared to cells lacking KIT D816V. Furthermore, ponatinib was found to inhibit the proliferation of primary neoplastic MC isolated from patients with indolent SM (ISM, n=4), ASM (n=2), and MCL (n=1), with IC_{50}-values ranging between 50 nM and 500 nM. Growth inhibition was accompanied by induction of apoptosis as assessed by light microscopy, flow cytometry, and TUNEL assay. Finally, we were able to demonstrate that ponatinib synergizes with midostaurin in producing growth-arrest and apoptosis in HMC-1.1 cells, HMC-1.2 cells, and in primary neoplastic MC isolated from a patient suffering from MCL. Synergistic TKI effects obtained with suboptimal concentrations of single agents were accompanied by complete blockage of important signaling molecules including KIT, Lyn, and STAT5. **Conclusions:** Ponatinib exerts major growth-inhibitory effects on neoplastic MC. Moreover, ponatinib synergizes with midostaurin in inducing growth inhibition and apoptosis in HMC-1 cells and primary neoplastic MC. Whether this drug-combination also exerts major antineoplastic effects *in vivo* in patients with ASM and MCL remains to be determined.

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IMPROVED EFFICACY OF MAB 37.1, A NOVEL FC-ENGINEERED CD37 ANTIBODY, IN COMBINATION WITH CHEMOTHERAPY AND RITUXIMAB IN MODELS OF B-CELL LYMPHOMA

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Background. MAb 37.1 is a CD37-specific antibody engineered for high-affinity binding to Fc-receptors with potent pro-apoptotic and enhanced ADCC activity against lymphoma cell lines and primary CLL cells. MAb 37.1 has substantially greater B-cell depleting activity in several *in vitro* systems than rituximab, and displays profound pharmacodynamic and anti-tumor effects in animal models. The antibody is currently in phase I clinical development for patients with B-cell malignancies. **Aims.** In clinical practice, the combination of B-cell depleting monoclonal antibodies (e.g. the CD20 mAb rituximab) with chemotherapy (e.g. CHOP, ICE) results in substantial benefit for patients with B-cell malignancies. To assess the potential of mAb 37.1 for combination with established chemotherapy regimens or rituximab, *in vitro* and *in vivo* studies were performed using the B-cell lymphoma lines Ramos and Raji. **Methods.** The effect of a combination of mAb 37.1 with CHOP or ICE was assessed in a nude mouse model with established Ramos tumors. MAb 37.1 was administered twice weekly at suboptimal doses (1 mg/kg). For CHOP chemotherapy a mixture of cyclophosphamide, doxorubicin and vincristine was injected i.v. once weekly, prednisolone was administered p.o. 5-times weekly. For ICE chemotherapy, a mixture of ifosfamide, carboplatin and etoposide was injected i.p. three times weekly on consecutive days. The pro-apoptotic effect of a combination of mAb 37.1 with chlorambucil and bendamustine was assessed *in vitro* on Ramos and Raji cells. Cells were incubated for 24 hours with different concentrations of chlorambucil (0 µM to 300 µM), bendamustine (100 µM to 400 µM), mAb 37.1 (10 µg/ml), or a combination thereof. Apoptosis was determined by FACS analysis of AnnV/PI positive cells. The combination of mAb 37.1 and rituximab was tested on Ramos cells. One antibody at a fixed concentration (mAb 37.1: 10 µg/ml; rituximab: 30 µg/ml) was combined with the other antibody titrated in a concentration range from 0.1 ng/ml to 10 µg/ml. **Results** In two independent experiments, mAb therapy as well as chemotherapy resulted in significant lymphoma growth delay *in vivo* (TGI mAb 37.1: 63%, CHOP: 74%; mAb 37.1: 70%, ICE: 65 %). The combination of mAb 37.1 and chemotherapy resulted in slower tumor growth (TGI mAb 37.1 + CHOP: 86%; mAb 37.1 + ICE 84%) indicating superior antitumor efficacy of the combination treatment. Treatment of Ramos or Raji cells with mAb 37.1, chlorambucil, or bendamustine *in vitro* resulted in induction of apoptosis. Combination of mAb 37.1 with chlorambucil or bendamustine resulted in a significant increase of apoptotic cells compared with single agent treatment, indicating a synergistic effect. Likewise, apoptosis induction by the combination of mAb 37.1 with rituximab exceeded that of single agent treatment, indicating an additive pro-apoptotic effect. **Conclusions.** Combination of the novel, Fc-engineered CD37 antibody mAb 37.1 with chemotherapy or rituximab resulted in improved efficacy against lymphoma cells *in vitro* as well as in a xenograft model of human lymphoma in mice. These data support further clinical evaluation of mAb 37.1 in combination with established treatment regimens in patients with B-cell malignancies.

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GAS1 AND KIF27 GENES ARE STRONGLY UP-REGULATED BIOMARKERS OF HEDGEHOG INHIBITION (PF-04449913) ON LEUKEMIA STEM CELLS IN PHASE I ACUTE MYELOID LEUKEMIA AND CHRONIC MYELOID LEUKEMIA TREATED PATIENTS

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Hedgehog (Hh) pathway activation contributes to leukemia development and growth, and that targeted pathway inhibition is likely to offer an efficient therapeutic opportunity. PF-04449913, a Hh pathway inhibitor, is a new selective and potent inhibitor of leukemia self-renewal and is currently being evaluated in phase I clinical trials. In order to identify new potential clinical biomarkers for the PF-04449913, we studied CD34+ leukemia stem cell population (LSC) collected before and after 28 days treatment in a phase I dose escalation protocol (Clinical Trial Gov. NTC00953758) enrolling selected hematological malignancies. This experimental clinical trial enrolled Myelofibrosis (MF), MDS, blastic phases CML, chronic myelomonocytic leukemia (CMML) and AML patients (pts). We were able to collect and separate highly purified (98%) bone marrow CD34+ cells from 5 AML, 1 MF and 2 CML pts by immunomagnetic separation, and analysed them for gene expression profile using Affimetrix HG-U133 Plus 2.0 platform. 1197 genes were differentially expressed between CD34+ cells collected before and after 28 days of PF-04449913 dose finding oral therapy. Clustering of their expression profiles showed that mostly genes differentially expressed are mainly related to Hh signaling,this providing further evidences that PF-04449913 really therapeutically targets the Hh pathway. Regarding genes involved in Hh signaling pathway, Gas1 and Kif27 were strongly upregulated (fold change 1.0947 and 1.12757 respectively; p-value 0.01 and 0.02 respectively) in CD34+ LSC after 28 days exposure to PF-04449913 as compared to baseline, suggesting these two genes have potential as new biomarkers of activity. GAS-1 is a Sonic Hedgehog (Shh)-binding protein; it acts to sequester Shh and inhibit the Shh signalling pathway. Kif27 mainly acts as a negative regulator in the Hh signaling pathway, and inhibits the transcriptional activator activity of Gli1 by inhibiting its nuclear translocation. Other genes were differentially expressed after 'ex- vivo' treatment with PF-04449913 as compared to baseline: we observed a down regulation of Bcl2 (fold change -1.03004), ABCA2 (fold change -1.08966), LEF1 (fold change -1.28457), Gli1 (fold change -1.0775), Smo (fold change -1.07702), and an upregulation of Gli2 (fold change 1.08191). Conclusions: This data demonstrates that PF-04449913 specifically targets the Hh Pathway in CD34+ cells, suggesting that Hh inhibition may impair leukemia stem cell maintenance. In addition, we identify several new potential biomarkers (e.g. Gas1 and KIF27). Taken together, these data may be useful for pts selection strategies and subsequent eradication of the LSC. Acknowledgments. Work supported by Pfizer, European LeukemiaNet, FIRB 2006, AIRC, AIL, COFIN, University of Bologna and BolognAIL.

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TOWARDS PERSONALIZED MEDICINE USING A NOVEL PRECLINICAL MODEL: IDENTIFYING DARATUMUMAB AS EFFECTIVE TREATMENT FOR MULTIPLE MYELOMA

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Multiple myeloma (MM), one of the most common hematological malignancies in adults, is a neoplasm of terminally differentiated B cells, i.e. plasma cells. The transition of a plasma cell to a fully transformed, aggressive myeloma is a multistep process, which requires subsequent acquisition of mutations in multiple genes. Most of this evolution takes place in the bone marrow (BM). Studying the pathogenesis of MM is seriously hampered by the lack of appropriate conditions for the engraftment of patient-derived MM (pMM) cells which, unlike MM cell lines, strongly depend on a human microenvironment to engraft, survive and expand. This indicates that the interaction of MM cells with the cellular and extracellular components of the human BM microenvironment plays a crucial role in the growth behavior of MM cells.Previously, we presented a unique mouse model to study the pathobiology of MM by implementing a technology for creating a natural human bone environment in the immune deficient $RAG2^{-/-}gc^{-/-}$ mouse. This humanized environment was found to act as a 3-D hematopoietic "niche"and to facilitate pMM cell growth, which was accompanied by bone resorption, being one of the most important clinical sequelae of MM. By gene-marking of pMM cells with luciferase and using bioluminescent imaging, we were able to follow myeloma outgrowth in time, and to visualize the effect of treatment. Using this novel model, we now have evaluated treatment responses of mice inoculated with pMM cells, by applying similar treatment as the MM patients received. Interestingly, the pMM-bearing mice showed identical responses as patients to the various treatments given. Indeed, mice receiving cells from heavily treated and refractory patients did not respond to treatment, while mice receiving cells from newly diagnosed patients did respond. In addition, pMM-bearing mice were used to further investigate the killing of MM cells by daratumumab (DARA).DARA is a human CD38 antibody with broad-spectrum killing activity. DARA effectively kills target cellsby CDC, ADCC and by induction of apoptosis, which is considered to be highlyrelevant for the treatment of hematological malignancies,including MM and chronic lymphocytic leukemia. Our data show that DARA was well able to kill MM cells from newly diagnosed patients in this model. More important, DARA was also effective on cells growing in the humanized ossicles and that were obtained from patients that were refractory to dexamethasone and bortezomib. Hence, our novel humanized mouse model can be utilized for preclinical testing, accurately visualizing treatment responses and resistance to therapy, and it confirms that DARA is an effective new treatment option for multiple myeloma patients.

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PRECLINICAL ACTIVITY OF THE JAK1/2 INHIBITOR RUXOLITINIB ON MALIGNANT PLASMA CELL GROWTH AND SURVIVAL

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Background. In multiple myeloma, cytokines in the tumor environment, in particular interleukin-6 (IL-6), support the growth and survival of malignant plasma cells. Binding of IL-6 to its receptor leads to gp130 dimerization and activation of Janus kinases (JAK) and STAT3 transcription factors. Ruxolitinib (INCB018424/INC424) is the first small molecule JAK inhibitor approved for the treatment of patients with myelofibrosis.**Aims.** The aim of our study was to evaluate the effects of ruxolitinib on malignant plasma cells. **Methods.** Cell growth was studied in seven human plasma cell lines including the IL-6 dependent INA-6. The JAK inhibitor was tested in a concentration range of 0.0625 µmol/L to 8 µmol/L. Proliferation of plasma cell enriched patient samples was measured by [3]H-thymidine uptake. IC50 concentrations and combination index were calculated with CalcuSyn. Apoptosis was evaluated by flow cytometry upon annexin(Ax)V-FITC/7-AAD staining. Levels of STAT3 and ERK1/2 phosphorylation were determined by Westernblot analysis. IL-6 levels were determined by ELISA.**Results.** Ruxolitinib induced a significant and dose-dependent inhibition of plasma cell growth in IL-6 dependent INA-6 cells with an IC50 of 0.22 µmol/L. Complete growth inhibition was achieved at 1 µmol/L in the absence and presence of bone marrow stromal cells. Importantly, stromal cell viability and IL-6 production were not affected. Treatment of INA-6

cells with 1 µmol/L ruxolitinib increased the number of apoptotic, AxV-positive cells from approx. 10% in cultures with medium alone to 39% and 63% after 48 and 72 hours, respectively. The induction of cell death is consistent with the inhibition of IL-6 induced STAT3 phosphorylation. A similar strong inhibitory activity of ruxolitinib (IC50 0.16 µmol/L) was observed in tumor cells of a patient with plasma cell leukemia which proliferated in response to IL-6. In addition, IL-6 dependent growth of B9 hybridoma cells was equally inhibited with an IC50 of 0.6 µmol/L of the drug. In contrast, none of the myeloma cell lines that grow autonomously were sensitive, pointing to the kinase specificity of the drug. Using INA-6 as a model, combinations with other signaling inhibitors revealed additive to synergistic effects with PI3K, mToR and IGF-1R inhibitors.**Conclusions:** In multiple myeloma, ruxolitinib has a strong cytotoxic activity against malignant plasma cells that require IL-6 for growth and survival. This warrants further clinical testing but also points to the need of identifying molecular markers to predict benefit from JAK inhibitor treatment.

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ERUFOSINE (ERUCYLPHOSPHO-N,N,N-TRIMETHYLPROPYLAMMONIUM) ALTERS THE EXPRESSION PROFILES OF MICRORNAS IN HUMAN ACUTE MYELOID LEUKEMIA CELL LINES

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Introduction. There is a clear need for new effective drugs in the treatment of acute myeloid leukemia (AML). Alkylphosphocholines are promising antitumor agents, which affect multiple signal transduction networks, inducing apoptosis and cell cycle block. Erufosine (erucylphospho-N,N,N-trimethylpropylammonium, ErPC3) is without significant toxicity to normal haematopoietic progenitors, therefore being very promising in leukemia treatment. The exact mechanisms underlying the antitumor activity of ErPC3 have not been completely elucidated. **Aims.** To evaluate the antileukemic potential of ErPC3 in AML cell lines and to estimate the impact on the expression of miRNAs. **Methods.** In vitro experiments were performed using AML cell lines: EOL-1 (ACC 386), Nomo-1 (ACC 542) and U-937 (ACC 5) [DSMZ]. For each cell line the cytotoxicity of ErPC3 was measured by the colorimetric MTT-assay and IC_{50} values after 24-48-72h incubation were calculated. In addition, a bead-based liquid assay for single-tube detection of 50 pre-selected on the basis of a known relation to AML miRNAs was conducted with total RNAs extracted from the studied cell lines before and after 48h incubation with erufosine at IC_{50} concentrations [Flexmir v.2, Luminex Corp.]. All samples were run in duplicates. The mean expression value for each microRNA in each sample was background. corrected and normalized to the untreated control. In addition, Western blot analysis was performed to investigate phosphorylated Akt (pAKT), JNK and Lamin B.**Results.** To assess whether ErPC3 affects the viability of AML cell lines, cells were treated with various doses (0,39-100 µM) for 24, 48 and 72h, harvested, and analyzed by MTT assay. Erufosine was found to have dose-dependent cytotoxicity in all studied cell lines with the lowest IC_{50} value of 2,2 µM after 72h of treatment in EOL-1, followed by Nomo-1 (IC_{50}=2,9 µM), while the relatively least sensitive cell line was U-937 (IC_{50}=4,76 µM). The multiplex analysis of the responses of miRNAs to ErPC3 treatment showed that mir-126, known for its tumor suppressor characteristics, was the only significantly up-regulated miRNA in all studied samples. On the other hand, the number of down-regulated miRNAs was related to the differences in the treatment efficacy, as the least sensitive U-937 cell line did not show any down-regulated miRNA, while the number of down-regulated miRNAs increased with the increase of chemosensitivity to ErPC3. The following six microRNAs were down-regulated in both of the remaining cell lines: mir-181a-5p, mir-17-5p, mir-92a-3p, mir-221-3p, mir-222-3p, mir-191-5p. On analyzing next the predicted targets and affected pathways of these altered miRNAs, MAPK, JNK, Akt were important molecular signaling pathways related to their expression patterns, which have also been previously shown to play central role in the mechanisms of alkylphosophocholines antitumor effects. This was confirmed by Western blot analysis showing down-regulation of pAkt, JNK alteration and lamin B fragmentation.**Conclusions:** These observations suggest that modulation of miRNA expression may be an important mechanism underlying the biological effects of erufosine and warrants further investigation of the targeted genes and signaling pathways.**Acknowledgements:** This work was supported by the National Science Fund and the Alexander von Humboldt Foundation.

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BOTANICAL ALKYL HYDROQUINONE HQ17(3) INDUCES REACTIVE OXYGEN SPECIES AND EXHIBITS CYTOTOXIC EFFECT ON RS4;11 ALL CELLS HARBORING T(4;11) CHROMOSOME TRANSLOCATION

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Background. Acute lymphoblastic leukemia (ALL) is the most prevalent childhood cancer. Although the rate of success in the treatment of childhood ALL has been increased to more than 80 percent for children and approximately 40 percent for adults with ALL, patients succumbing very-high- risk (VHR) ALLs (those harboring t(9;22) or MLL rearrangements chromosomal abnormalities) display poor clinical outcomes even with intensive combined chemotherapies. HQ17(3) (10'(Z), 13'(E), 15'(E)-heptadecatrienyl-hydroquinone), is a natural product isolated from the sap of *Rhus succedanea*. HQ17(3) has been reported to have cytotoxic activity on tumor cell lines (cervical, colon cancers and hepatoma cells) and HL-60 myeloid leukemia cells. We found HQ17(3) exhibited very effective cytotoxic effect on four tested ALL cell lines (RS4;11, REH, SUP-B15, and JURKAT) with the IC_{50} of 0.5 to 5 µM, but spared normal peripheral blood mononuclear cells. Thus, investigation of the molecule mechanisms involved in the selective cytotoxic effects on leukemic cells will be beneficial for finding new anti-leukemic therapeutics. **Aims.** To investigate the molecular pathways involved in the HQ17(3)-induced cytotoxicity in acute lymphoblastic leukemia cells RS4;11 (VHR ALL harboring t(4;11)). **Methods.** HQ17(3)-treated and control RS4;11 cells were subjected to the following tests to study the cytotoxic effects of HQ17(3) on lymphoblastic leukemic cells. The membrane lipid disturbance in apoptotic cell death was analyzed by Annexin V/PI stain. DNA fragmentation was defined as sub-G1 fraction of cellular DNA content after the PI staining. ROS production and mitochondrial membrane potential loss were stained by DCFDA and DiOC6(3), respectively. The stained cells were subjected to flow cytometric analysis. Western blot analysis was applied to study the activation of caspases 3, 7, 9 and cleavage of poly (ADP-ribose) polymerase (PARP) after HQ17(3) treatment. **Results.** RS4;11 cells treated with HQ17(3) showed features of apoptotic cell death (Annexin V^+ and DNA fragmentation) in 24 hours. HQ17(3) induced reactive oxygen species (ROS) production and disrupted the mitochondrial membrane potential. Activated caspase-3, and cleaved PARP were confirmed. Pan-caspase inhibitor, zVAD-fmk, effectively inhibited HQ17(3)-induced caspase-3 activation, however, could not rescue cells from death. Thus, HQ17(3) induced caspase-independent cell death of RS4;11 cells. Addition of antioxidants or ROS scavengers (GSH or vitamin C) attenuated HQ17(3)-induced ROS production, mitochondrial membrane potential lost, and cell death. These results indicate that oxidative stress associated with ROS production be the major mechanisms implicated in HQ17(3)-induced RS4;11 cell death.**Summary/conclusion:** Naturally-derived HQ17(3) displayed significant anti-leukemic activity in RS4;11 ALL cells by inducing ROS production and apoptotic cell death. It is interesting to further investigate molecules implicated in HQ17(3)-induced, caspase-independent DNA fragmentation and cell death. These results further indicated that agents selectively induce or sustain ROS in leukemic cells would potentially augment the treatment for VHR ALL with t(4;11) translocation.

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FOCUS ON INTRACELLULAR SIGNALING - NEW TARGETED THERAPEUTIC APPROACHES IN DIFFUSE LARGE B-CELLS LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) is a common type of non-Hodgkin lymphoma, accounting for about 25-30% of all cases in the western countries. It is a clinically aggressive lymphoma in which the patients have a five-year survival rate of 50%. Several intracellular pathways are related to lymphomagenesis and of the most frequently involved are the BCR/PI3K/AKT/mTOR pathway (B-Cell-Receptor/Phosphatidylinositol-3-kinase/AKT protein kinase B/Mammalian Target of Rapamicin), NF-kB pathway (Nuclear factor kB) and RAS/MAPK pathway (MAPK, Mitogen Activated Protein Kinase, associated with the RAS-RAF proteins). RAS proteins are activated by farnesylation, a process mediated by the farnesyltranferase enzyme. On the other hand, several proteins involved in these pathways are degraded in the ubiquitin-proteasome pathway.In this context, the aim of this study is to evaluate the potential therapeutic of the mTOR inhibitor (Everolimus), the proteasome inhibitors (MG262), the IkB inhibitior (Parthenolide) and the farnesiltransferase inhibitor (L-744832) in DLBCL.For this purpose we used the a DLCL cell line, the Farage cells, cultured in the absence and presence of several concentrations of Everolimus, MG262, Parthenolide and of L-744832, in monotherapy and in association with each other and with conventional chemotherapy drugs (Vincristine, VCR). Cell growth and viability were evaluated

by the rezasurin assay. The effectiveness of the drugs was determined by dose-response curve with determination of IC_{50}. Cell death was analyzed by optical microscopy using the May-Grunwald staining and by flow cytometry, through the annexin-V and propidium iodide double staining. We also analyzed, by flow cytometry, cell cycle using Propidium Iodide incorporation and the expression levels of KI-67, Lamin A/C, Ubiquitin conjugates, NF-kB and Caspase 3.Our results show that all of the tested compounds induced cell death in a time- and dose-dependent manner, with IC_{50} values of 25 μM for Everolimus, 25 nM for MG262, less than 10 μM for Parthenolide and ranging from 50 μM to 75 μM for L-744832, after 24h of treatment. These compounds induced cell dead mainly by apoptosis, which may be mediated by caspase 3. These drugs also induce cell cycle arrest in G0/G1 phase, mainly Everolimus, which may be related with a reduction of KI-67 and Lamin A/C levels and with an increased in ubiquitin conjugates levels. The association of lower doses of these inhibitors shows an additive/synergist cytotoxic effect.In summary, this study suggests that Everolimus, MG262, Parthenolide and L-744832 alone or in combination with VCR, may constitute new promising therapeutic approach in DLCL treatment.*Frist and second authors contribute equally to the study.*

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THE ANTI-KAPPA MONOCLONAL ANTIBODY MDX-1097 COOPERATES WITH IMMUNOMODULATORY DRUGS TO ENHANCE ANTIBODY-DEPENDENT CELL CYTOTOXICITY OF MULTIPLE MYELOMA CELLS

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Background. Multiple Myeloma (MM) is a malignancy of clonal plasma cells in the bone marrow with median overall survival duration of 3-5 years. Despite recent advances in the treatment and management of MM, which have improved progression free survival (PFS) and overall survival (OS), the majority of patients will ultimately relapse and die from their disease. The anti-kappa monoclonal antibody, MDX-1097, is one of many novel therapeutic approaches in development for the treatment of MM. MDX-1097 currently is being assessed as a single agent in a Phase 2 clinical trial for the treatment of kappa light-chain restricted (κ-type) MM. MDX-1097 binds to kappa myeloma antigen (KMA), a tumor-specific membrane-associated protein expressed on malignant plasma cells in patients with κ-type MM and exerts its anti-tumour effects via a number of mechanisms including antibody-dependent cell cytotoxicity (ADCC) in the presence of human immune effectors such as natural killer (NK) cells. Immunomodulatory drugs (IMiDs) such as lenalidomide, are currently in use for the treatment of MM and have been shown to exert anti-tumor effects both directly, via apoptotic mechanisms, and indirectly via a number of different mechanisms including the augmentation of NK-dependent cellular cytotoxicity. **Aims.**To determine whether IMiD-treated PBMCs more effectively kill MDX-1097-bound MM cells via ADCC compared to untreated PBMCs, and whether IMiD-treated MM cells are more sensitive to MDX-1097 mediated immune effector cell cytotoxicity compared to untreated MM cells. **Methods.** For ADCC assays, a FACs-based method was used. Briefly, untreated or IMiD-treated JJN3 cells (a κ-type MM cell line) were first labeled with carboxyfluorescein succinimidyl ester (CFSE) and then incubated with MDX-1097 or human IgG. These JJN3 cells were mixed with untreated or *in vitro* IMiD-treated PBMCs from normal donors, or with PBMCs from MM patients pre- and post-IMiD treatment. 12 hours later, the viability dye 7-AAD was added to the cells and analyzed by FACS. Percentage cell death was calculated as the proportion of CFSE+/7-AAD+ cells to the total number of CFSE+ cells. **Results***In vitro* pre-incubation of normal PBMCs with IMiDs prior to incubating with MDX-1097 treated JJN3 cells caused 1.5 fold more cell death, compared to untreated PBMCs. Pre-incubation of JJN3 cells with IMiDs resulted in a 2-fold increase in KMA expression (and MDX-1097 binding) and enhanced ADCC by 1.7 fold in the presence of untreated PBMCs when compared to non IMiD-treated JJN3 cells. A further modest increment in cell killing was observed when IMiD-treated PBMCs were mixed with IMiD-treated JJN3 cells. Finally, use of *in vivo* IMiD exposed PBMCs isolated from a MM patient treated with IMiD demonstrated that these PBMCs were, on average, 1.8 fold more effective in killing MDX-1097 bound JJN3 cells compared to PBMCs obtained from the same patient prior to IMiD treatment. **Summary/Conclusions**Our *in vitro* results suggest that treatment of PBMCs and MM cells with IMiDs can enhance MDX-1097-mediated ADCC of MM cells. This provides a strong rationale for the clinical evaluation of MDX-1097 in combination with IMiDs for the treatment of multiple myeloma.

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OCARATUZUMAB, A FAB-ENGINEERED ANTI-CD20 ANTIBODY, DEMONSTRATES GREATER AFFINITY TO CD20 AND ABILITY TO BIND TO RITUXIMAB-COATED B-CELLS

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Background. Ocaratzumab, previously known as AME-133v, is a humanized, Fab-engineered anti-CD20 antibody optimized for superior binding. Using single-point codon-substitutions, thousands of variations of the antibody complementary determining regions were screened. Ocaratuzumab was selected from the variants because of its improvement in K_{off} rate, which prolongs binding of the antibody. Compared to the K_d constant of rituximab, ocaratuzumab has 13- to 20-fold greater binding affinity for CD20 (Figure 1). Ocaratuzumab and rituximab bind to the same epitope, potentially allowing competition and displacement between the two antibodies.**Aims.** Experiments were performed to assess the ability of ocaratuzumab's increased affinity to improve binding to lymphoma B-cells pre-coated with rituximab. **Methods.** 50 μg/mL of biotinylated rituximab was added to fixed SKW.4 lymphoma B-cells and incubated overnight at 37°C. Ocaratuzumab was added to each plate at half the concentration of rituximab (25 μg/mL) for 3, 6, and 24 hours. The amount of rituximab still bound to the B-lymphocytes was assessed by detecting biotinylated antibody using neutraAvidin alkaline phosphatase conjugate and a colorimetric substrate (optical density was measured at 560 nm in a spectrophotometer).**Results.** Even at half the concentration of rituximab, ocaratuzumab reduced the amount of rituximab bound to the lymphoma B-cells. The reduction in pre-bound rituximab was 8.44% at 3 hours, 16.32% at 6 hours, and peaked at 31.12% at 24 hours from baseline. Additional experiments were performed, and higher concentrations of ocaratuzumab (75, 150 μg/mL) showed no additional displacement of rituximab, suggesting that low concentrations of the antibody are effective in maximally displacing rituximab bound to B-cells. Under controlled conditions, no spontaneous reduction in rituximab binding occurred, demonstrating the ability of ocaratuzumab to preferentially replace rituximab due to its faster K_{on} rate and its ability to remain bound longer due to its slower K_{off} rate. Furthermore, independent experiments have shown pre-incubation of ocaratuzumab in primary chronic lymphocytic leukemia cells can significantly inhibit the binding of rituximab. This suggests that the improved affinity of ocaratuzumab to CD20 and its low K_{off} rate prevent binding of a second antibody to these B-cells.**Conclusions:** Ocaratuzumab (AME-133v) has the ability to bind to rituximab pre-exposed B-lymphocytes even at concentrations 50% lower than that of rituximab. Combined with Fc-engineering to improve antibody dependent cellular cytotoxicity, ocaratuzumab may improve clinical benefit in rituximab pre-treated patients with B-cell neoplasia.

Table 1. Ocaratuzumab and Rituximab Binding Kinetics on Lymphoma Cells.

	Ocaratuzumab	Rituximab
K_d (pM)	97	2097
K_{on} (10^5/M/s)	7.8	4.7
K_{off} (10^{-5}/s)	7.6	98.6

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LASRT (LARGE-SCALE REAL-TIME TITRATION): A RECOMBINED VIRUS TITRATION METHOD SIMPLE, SAFE AND EFFICIENT FOR PRE-CLINIC GENE TRANSFER RESEARCH

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Background. Titer of recombinant virus (RV) on cells is the most accurate and important index, however, classic titration method need to be improved. If RV can infect human cells, titration should be carried out in S2 lab, but method used for titering such as flow cytometry (FCM) is normally not carried in S2 condition in most labs. Exposure to the environment means either much more works to exclude any infective virus in the samples or to detect at high risks. Is it possible to simply detect the titers under S2 condition? **Aims.** To establish and evaluate a simple, safe and efficient titration method (LaSRT) for pre-clinic gene transfer research **Methods.** :(1)Virus: Lentivirus with GFP maker was produced by pWPXLd, pMD2.G, pCMVR8.74 3-vector-system (Addgene Co.) and 293T/17 cell (ATCC). Retrovirus with puromycin resistant gene was produced by pMSCVpuro vector (Clontech Co.) and Plat-E cell (Cellbiolabs Co.). 293 cells (ATCC) are used as titration target cell. Virus supernatants from 48h to 72h were used. (2) Classic titration: For lentivirus with GFP, FCM titration was used as control. 2 × 10e5 293 cells were transfected with serial dilutions of supernatant as 1mL, 100μl and 10μl (n=3). Titer (TU/mL)=(2×10e5 target cells)×(positive cells%)/volume of supernatant (mL). For pMSCVpuro retrovirus, 2-week-clone-forming method were used as control.(3) LaSRT titration (see Fig.1): ① -1d, plate 10 000 293 cells/well in a 96 well-plate, each well with 180ul IMDM, 10% FCS and 4 μg/mL Protamine sulfate (Elkins-Sinn, Inc.); ② 0d, add 20ul virus supernatant to the first well then to limited dilute at 1:10 in the following wells (n=3), ③+2d, observe GFP$^+$ cells under inverted fluorescence microscope, the positive cell numbers (M) would only be counted in the "counting well"(the last well where the M≤10, and no positive cells in the next well), the serial number (N) of the counting well is used for titer calculation.④ Titer Transfection Units (TU/mL) = M×10^{N+1}.⑤ For pMSCVpuro retrovirus, the only difference is to count survival cells in the counting well on +4d.4. Results. No significant difference was seen between the results of pWPXLd GFP-lentivirus got by FCM vs LaSRT, (5.3±1.5)×10e5vs(5.1±1.3)×10e5,P>0.05; as well as between those of pMSCVpuroR retrovirus got by CF vs LaSRT, (7.0±4.4)×10e5vs(5.8±3.7)×10e5,P>0.05. 5 Summary / ConclusionsLaSRT is a better titration method for pre-clinic gene transfer reasearch for the following reasons: ①Simple and accurate, regardless big difference of original titers of RVs; ② Safe□closed system till detection; ③Efficient and economic, large scale samples can be tested with only a drop of virus; ④Valid for different RV with a suitable maker, such as AV, AAV and LV with GFP. In near future, it can be easily developed to be automatic or semi-automatic detection as RQ-PCR and FISH.

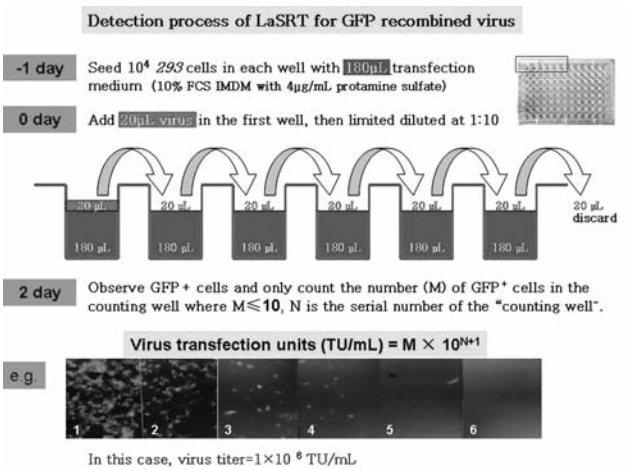

Figure 1 Detection process of LaSRT for GFP recombined virus.

Quality of life and health economics - Multiple Myeloma

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THE IMPACT OF TREATMENT PERSISTENCE WITH LENALIDOMIDE FOR THE TREATMENT OF MULTIPLE MYELOMA (MM) ON DISEASE CONTROL

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Background. While persistence to drug therapy is essential to achieve optimal patient benefits, poorly controlled MM results in more rapid disease progression, disease-related complications, impact on quality of life, and ultimately death.**Aims.** To evaluate the real-world persistence with lenalidomide treatment and to assess the relationship between treatment persistence and indicators of lack of disease control and disease-related complications for MM patients. **Methods.** Claims database from aUS health plan were assessed between 1 July 2007 and 30 June 2011 to identify commercial enrollees and Medicare Advantage enrollees with Part D (MAPD), initiating lenalidomide for treatment of MM. MM was identified by at least 2 medical claims at least 7 days apart with a MM diagnosis code (ICD-9: 203.0x). Eligible patients were required to have continuous pharmacy and medical benefits in the 6 months prior and 1 year following initiation of lenalidomide. Treatment discontinuation was defined as the first appearance of a gap greater than 30 days between runout date (prescription fill date + days supply) and next lenalidomide prescription fill. Persistence was defined as the number of days from the first date of treatment with lenalidomide to the earlier of the date of discontinuation or end of the 12-month follow-up period. Indicators of disease control included in this study were an inpatient hospitalization and number of ER visits. Disease-related complications included in this study was evidence of skeletal-related events (SREs) defined as fracture, spinal cord compression, or radiation to the bone; and sepsis after initiation of lenalidomide. Logistic and negative binomial regression models were used to examine the relationship between persistence and measures of disease control and complications, controlling for age, sex, comorbidity score, prior stem cell transplant, prior SREs, and insurance type (commercial or MAPD).

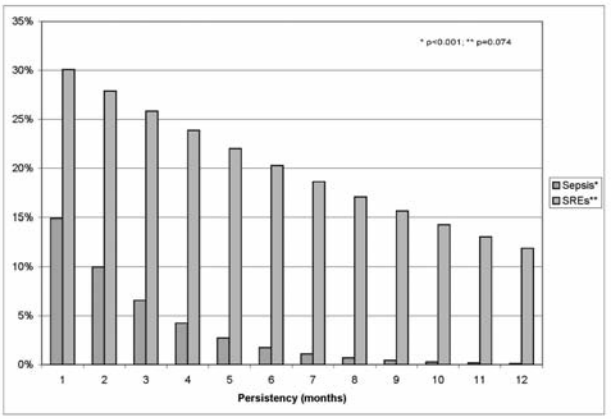

Figure 1. Predicted Risk of Sepsis and SREs as a function of lenalidomide persistence.

Results. 1,524 lenalidomide-treated MM patients were identified; 605 had at least 12 months of continuous eligibility following lenalidomide treatment initiation, 690 disenrolled from the health plan prior to 12 months, and 229 died prior to 12 months. Among the 605 patients enrolled for 12 months, persistency with lenalidomide averaged 6.0 months (median = 4.9) with 350 (57.9%) persistent for the entire year. Sixty-six (10%) of patients developed sepsis and 167 (28%) developed SREs. Controlling for the variables listed above, a one-month increase in persistence/treatment duration was found to be associated with, on average, a lower probability of developing SREs (OR=0.88; p-value=0.074) as well as sepsis (OR=0.63; p-value<0.001) [Figure 1]. Additionally, the probability of an inpatient hospitalization (OR=0.68; p-value<0.001) and additional ER visits (OR=0.83; p-value=0.002) were both lower with better persistence. **Conclusions.** Greater persistence with lenalidomide therapy is associated with improved patient outcomes as demonstrated by fewer SREs and

lower likelihood of developing sepsis, consequently leading to fewer hospitalizations and ER visits. This analysis demonstrates that continuous treatment with lenalidomide may not only improve disease control in MM patients , but in addition also reduces health care utilization and related costs as indicated by the lower risk of a hospitalization and number of ER visits.

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HEALTH RESOURCE UTILISATION ASSOCIATED WITH SKELETAL-RELATED EVENTS IN MULTIPLE MYELOMA: A PROSPECTIVE MULTINATIONAL OBSERVATIONAL STUDY

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Background. Skeletal-related events (SREs; pathologic fracture, radiation to bone, spinal cord compression or surgery to bone) are common in patients with bone disease related to multiple myeloma and are associated with increased patient morbidity and mortality. Limited prospective data on the related economic burden are available in the literature.**Aims**To evaluate health resource utilisation (HRU) associated with SREs in patients with bone lesions secondary to multiple myeloma. **Methods.** Patients with bone lesions secondary to multiple myeloma; at least one SRE within 97 days prior to enrollment; life expectancy ≥6 months; ECOG 0, 1 or 2 were enrolled from Germany, Italy, Spain and the UK. Data on HRU associated with SREs were collected retrospectively for 90 days prior to enrollment and prospectively for up to 18-21 months. HRU type included number and length of inpatient hospitalisations, outpatient visits, emergency room visits and procedures. Attribution of HRU to each SRE was determined by the investigators. **Results.** A total of 153 eligible European (Germany n=51; Italy n=39; Spain n=38; UK n=25) patients with MM entered the study; mean age (SD) ranged from 63.7 (9.9) to 67.9 (10.0) years. Percentage of female patients ranged from 12.0% (UK) to 56.4% (Italy), Germany and Spain reported 41.2% and 44.7%, respectively. A total of 103 of 281 SREs (36.7%) were associated with in-patient stays with a mean (SD) duration of 18.1 (19.8) days per stay for the 114 stays (a SRE could contribute multiple hospitalisations). The length of inpatient stays varied by facility (i.e., oncology, radiation, surgical) and SRE type. The most common SRE requiring hospitalisation was surgery to bone: 34 of 47 events (72.3%) with 39 in-patient stays requiring a mean (SD) length of stay of 12.7 (14.4) days. The least common SRE requiring hospitalisation, radiation to bone (15 of 107 events [14.0%]), was still associated with 13 in-patient stays with an average (SD) of 18.3 (10.2) days per stay. Overall, 179 (63.7%) SREs required at least one outpatient visit and 88 (31.3%) required >5 visits. As expected, radiation to bone was associated with the highest number of outpatient visits: of the 107 SREs, 80 (74.8%) were associated with an outpatient visit. Surgery to bone and spinal cord compression were associated with the least number of outpatient visits, with 24 (51.1%) of 47 SREs and 16 (53.3%) of 30 SREs requiring a visit, respectively. Seven (2.5%) of 281 SREs (5 pathologic fractures and 2 spinal cord compressions) were associated with an emergency room visit. **Summary/Conclusions**SREs are associated with lengthy hospitalisations and numerous outpatient visits. Thus, preventing SREs may substantially reduce the burden of HRU across European healthcare systems.

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THE IMPACT OF NEW TECHNOLOGIES ON MULTIPLE MYELOMA MANAGEMENT: A COST-EFFECTIVENESS ANALYSIS

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Background. Although the introduction of high-dose therapy and novel agents has improved the outcome of MM in terms of response, progression free and overall survival (PFS, OS), it is still not clear if this amelioration has also produced a significant improvement in cost/effectiveness. **Aim**This study aimed to evaluate the economic impact of new technologies in Multiple Myeloma frontline treatment.**Methods**Two groups of patients were compared: Group A included 78 patients receiving MP between January 1th 1986 and December 31st 1994; Group B included 73 patients receiving an autologous transplant between January 1th 1995 and December 31st 2003. Patients were considered eligible for the study when having baseline characteristics suitable for transplantation whether or not high-dose therapy was an available treatment option at time of therapy starting.**Results**The characteristics of patients at presentation were similar in the two groups. After a median follow-up of 36.7 months and 50.7 months, 3% and 24% patients were alive in group A and B respectively. The median overall survival (OS) was 3.2 years and 5.4 years respectively (p=0.0002). Analysis of costs was done by computing resource utilization: number and cause of hospital admissions split by type of admission (outpatient visit, day-hospital, long-term hospitalization), type of administered therapies, concomitant treatments particularly focusing on supportive therapies (transfusions, growth factors, antibiotics, bisphosphonates). The resource costs were computed in accordance to the current "Sistema Sanitario Regionale (SSR)"regional tariffs.A total number of 1756 hospital admissions were distributed as follows: 778 in group A and 978 in group B. Hospital admission distribution was different between the two groups (p<0.001): 407/778, 52% long-term hospitalizations in group A vs 607/978, 62% in group B; 231 (29%) outpatient visits and 140 (19%) day-hospital admissions in group A vs 243 (25%) and 128 (13%) in group B.A cost utility analysis was carried out based on quality-adjusted life years (QALY). QALYs were computed by applying utilities to follow-up periods: 0.8 during off-treatment periods, 0.58 during therapy, and 0.63 during maintenance (values based on the literature). The incremental cost-effectiveness ratio (ICER) was calculated for a single patient.In order to make the two groups comparable in terms of length of observation, cases in group A were censored when exceeding the maximum observation time in group B.Mean cost per patient was significantly different between groups 9343 € in group A vs 103948 € in group B (p<0.001). The total person-years were 336,4 and 478.2 respectively in group A and B, while the cost per person-year was 5134.4 € and 9161.9 € in group A and B respectively. The final quality adjusted life-year gain in group B patients as compared to group A was 1.79 QALY, with an ICER of 52767€.**Conclusions**Basing on the significant prolongation of OS obtained with first-line autologous transplant with respect to conventional oral therapy (2,2 years P=0,0002) in a still poor prognosis disease, and keeping into account the low prevalence of multiple myeloma, our conclusion is that the calculated ICER is within an acceptable threshold.

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COST EFFECTIVENESS OF CHEMOTHERAPY REGIMENS IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: A SYSTEMATIC REVIEW

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Background. An important treatment goal for patients with newly diagnosed multiple myeloma (ndMM) is maintaining the level of response achieved through successful induction therapy. Results from clinical studies demonstrate that maintenance treatment may provide favorable quality of response, progression-free survival and overall survival. However, to facilitate successful market access, all new treatments require economic assessment to determine their cost-effectiveness to healthcare services.**Aims.** To conduct a systematic literature review of health economic evidence for maintenance therapy in patients with ndMM. **Methods.** A broad search strategy was devised to identify all cost-effectiveness data for multiple myeloma (MM) treatments both in ndMM and in patients with relapsed or refractory MM (rrMM), and in patients eligible for autologous stem cell transplantation (ASCT) and ineligible for ASCT. Searches were performed in Medline, MEIP, EMBASE and BIOSIS via OVID and in the NHS Economic Evaluation Database. Screening was performed in two steps. One reviewer screened and extracted the data, a second checked the screening and extracted data, and a third independent reviewer checked the report for accuracy. **Results.** Four manuscripts and six abstracts were identified, each of which reported the results for distinct studies.[1-10] Five of these studies were cost-effectiveness analyses (CEAs) of treatments in patients with ndMM, one was a cost-consequence analysis in patients with ndMM, and four were CEAs of treatments for patients with rrMM. No studies investigating the cost-effectiveness of maintenance therapy were identified. **Conclusions.** To date no studies have been published on the cost-effectiveness of maintenance therapy in ndMM, either following ASCT or following induction therapy in patients ineligible for ASCT. Currently there are no treatments approved for maintenance therapy in MM which may offer a reason for the absence of cost-effectiveness studies in this area. For treatments currently under regulatory review, cost-effectiveness evidence may be required for reimbursement. However, the accu-

racy of such assessments will be contingent upon the quality and extent of data available. [1]De Abreu Lourenco et al. ISPOR Oct 2009; [2]Green et al. HTA 2009;13(1):29-33; [3]Hornberger et al. Eur J Haematol 2010;85(6):484-91; [4]Joseph et al. ISPOR Oct 2009; [5]Liwing et al. ISPOR Oct 2009; [6]Porter and Rifkin Clin Lymphoma Myeloma 2007;7(4):S150-5; [7]Sampson et al. Br J Haematol 2001;113(4):1015-9; [8]Wang et al. ASH 2009; [9]Yoong et al. ISPOR Oct 2009; [10]Rickert et al. ISPOR May 2009.

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COST-EFFECTIVENESS OF LENALIDOMIDE/DEXAMETHASONE IN MULTIPLE MYELOMA PATIENTS WITH A PRIOR THALIMODMIDE THERAPY

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Background. NICE guidance recommends thalidomide or bortezomib for first-line treatment of multiple myeloma (MM), two agents associated with a risk of peripheral neuropathy (PN). PN can interfere with daily activities leading to treatment discontinuation (5-10% of patients) and dose reduction (approximately 15% of patients). For patients developing PN following first-line treatment, lenalidomide/dexamethasone (Len/Dex), approved by the EMA for patients with MM who have received ≥1 prior therapy, may be an appropriate option for second-line treatment. **Aims.** Economic modelling evaluated the cost-effectiveness of Len/Dex vs dexamethasone (Dex) from the perspective of the National Health Service (NHS) in England and Wales in patients previously treated with thalidomide. **Methods.** An Excel-based individual simulation model was developed for relapse refractory MM patients in the MM-009/10 trials who had prior thalidomide therapy to determine time-to-progression (TTP) and overall survival (OS) after being treated with Len/Dex or Dex. OS within the model was calculated from the sum of TTP and post progression survival (PPS) for each patient. The effect of treatment on TTP was derived from a subgroup analysis of patients from the MM-009/010 pivotal trials (Len/Dex vs Dex) who had received one prior therapy. PPS was also derived from the MM-009/010 trials; however, as 47% of patients on Dex switched to Len/Dex at progression, there was a significant crossover effect in the Dex group. This was adjusted for using data from the UK Medical Research Council myeloma trials. Resource use for monitoring and managing adverse events (AEs) was obtained via a survey of expert haematologists in the UK. Drug costs and costs for managing AEs were taken from NHS sources. A patient access scheme whereby patients received Len free of charge beyond 26 cycles was included. Utility values were taken from published literature and applied to the period prior to progression (based on best response achieved) and after progression. The robustness of model results was assessed by sensitivity analyses whereby individual model parameters were varied across 95% confidence intervals. Cost-effectiveness was determined over a lifetime horizon; a discount rate of 3.5% was applied for future benefits and costs. **Results.** Compared to Dex, Len/Dex was associated with a substantial increase in life years (LYs, 4.42) and quality-adjusted life-years (QALYs, 2.97) in patients who have received prior thalidomide therapy. Total costs were greater for Len/Dex than Dex (£79,549 vs £3,134), but were off-set by the gains in LYs and QALYs, resulting in a cost of £17,290 per LY gained and £25,713 per QALY gained. Model results remained robust to sen sitivity analyses conducted on most parameters. **Conclusions.** These results indicate that Len/Dex is a cost-effective option when compared to Dex as second-line treatment for relapsed/refractory MM patients who have received thalidomide as a prior therapy (<£30,000 per QALY gained). Len/Dex is currently recommended by NICE for MM patients with ≥2 prior therapies. However, recent guidance recommending thalidomide and bortezomib as first-line treatments suggest that Len/Dex may be an option for patients who relapse, or develop PN following first-line treatment.

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ECONOMIC IMPACT OF BENDAMUSTINE IN THE PHARMACEUTICAL EXPENDITURE IN THE TREATMENT OF MULTIPLE MYELOMA IN SPAIN

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Background. Due to the development of novel cytostatic agents such as proteasome inhibitors and immunomodulators, the standards of care for the Multiple Myeloma (MM) patient has changed. The costs associated with current and emerging therapies are significant and pose a tremendous financial burden to the Public Health Systems, specifically at the hospital setting. Bendamustine is a novel bifunctional alkylating agent recently available in Spain, approved for treatment of non-candidates for transplantation MM patients, which is under investigation to be combined with the other cytostatic drugs in relapsing-remitting MM patients. **Aims.** To calculate the economic impact of MM treatment in Spain, and to develop a Budget Impact Analysis (BIA) to estimate the potential savings bendamustine availability would introduce as a treatment option for MM patients, non-candidates for transplantation, who are likely to be treated with other cytostatic combinations. **Methods.** A Budget Impact model was developed, assuming a prevalent treatable population of 1,380 patients in 1st line and 2,317 patients in 2nd onwards lines in 2011, with a patient estimated increase at a 3% yearly rate. The time horizon considered was 3 years and the analysis was made from the perspective of the Spanish Health System. Pharmaceutical expenditure on cytostatic agents was analyzed, excluding other medical costs. The drug costs were based on the official ex-factory prices (EFP), discounting the price reduction established in 2010 by the Spanish Government. The base case scenario (without bendamustine) was based on prevalent epidemiological and market survey data available, enriched with the expert opinion input. The bendamustine scenario considered different drug combinations and different dosages (90-120mg/m^2), depending on the treatment line to analyze. No combinations bendamustine+lenalidomide were considered in the model due to the hematological toxicity profile of both drugs. A deterministic sensitivity analysis (SA) was performed, switching the most relevant variables included in the model, to confirm the BIA results robustness. **Results.** The drug expenditure for MM treatment is slightly under €130 Mio/year in Spain. There is a wide variety of drug acquisition costs, the single cycle costs are ranging from €3 (melphalan+prednisone) to €5,969 (lenalidomide+dexamethasone). Bendamustine based treatments would reach from 7% (1st year) to 21%(3rd year) of the treated patients analyzed. Fifty-four percent of the bendamustine treatments considered were combinations with other high-price drugs (bortezomib or thalidomide), where the bendamustine cost would be only 17% or 21% respectively of the whole treatment regimen. The introduction of bendamustine would reduce drug expenditure in €5.4 Mio/year on average. The SA confirmed the data analyzed, with savings ranging from €14.3 to €18.2 Mio in the 3-year cost projections. **Conclusions.** The economic impact of MM treatment in Spain poses a high financial burden to the hospital drug budgets. The introduction of bendamustine would produce drug cost savings in the MM patients, mainly from the 2nd treatment lines onwards. Considering only the drug acquisition costs, the savings observed in the model were mainly due to the substitution of high cost combinations like those based on bortezomib, thalidomide or lenalidomide therapies.

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MODELLING THE 10-YEAR COST EFFECTIVENESS OF BENDAMUSTINE AS FIRST LINE TREATMENT FOR CHRONIC LYMPHOCYTIC LEUKAEMIA IN THE NETHERLANDS

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Background. Although promising treatment combinations for chronic lymphocytic leukemia (CLL) like fludarabine, cyclophosphamide and rituximab (FCR) became available in the last decade, a significant proportion of patients is not suitable or eligible for such intensive chemotherapy. In those patients chlorambucil remains a widely used first-line therapy. However, overall and complete response rates with chlorambucil are relatively modest and therefore more effective treatment options are required for this patient group. Bendamustine is a promising drug in this setting, but by being more expensive compared to chlorambucil, it will increase pressure on health care budgets. In this situation, healthcare authorities will require information on cost-effectiveness for regulating reimbursement. **Aims.** The objective of this study was to assess the cost-effectiveness of bendamustine compared to chlorambucil, the current first line treatment for CLL patients with Binet stage B or C disease not eligible for fludarabine combination therapy, from a health care payer perspective in the Netherlands. **Methods.** A Markov model was designed representing the normal evolution of patients with CLL over different treatment lines (Figure 1). Transition probabilities were derived from clinical trials. Healthcare resource utilisation was estimated for each CLL state using clinical guidelines and a Dutch CLL expert panel. Outcomes were life years (LY), quality-adjusted life years (QALYs), progression free life years (PFLY), and CLL related health care costs (e.g. in- and outpatient visits, diagnosis tests, chemotherapy and immunotherapy, costs of best supportive care). The model time horizon was 10 years. **Results**. The mean number of QALYs was 3.77 for bendamustine and 2.21 for

chlorambucil. The total average costs amounted to €79,328 for bendamustine, and €67,172 for chlorambucil (2011 values). Compared with chlorambucil, the cost-effectiveness of bendamustine was €7,809 per QALY gained. The costs per LY gained were €7,374 and the costs per PFLY gained were €6,908. The probability is around 95% that bendamustine costs less than €20,000 per QALY when compared with chlorambucil.Conclusions. Bendamustine compared to chlorambucil, in previously untreated CLL patients with Binet stage B or C disease not eligible for fludarabine combination therapy, generated an ICER of €7,809 per QALY gained, indicating that bendamustine is 10-year cost-effective as first line treatment for CLL in the Netherlands.

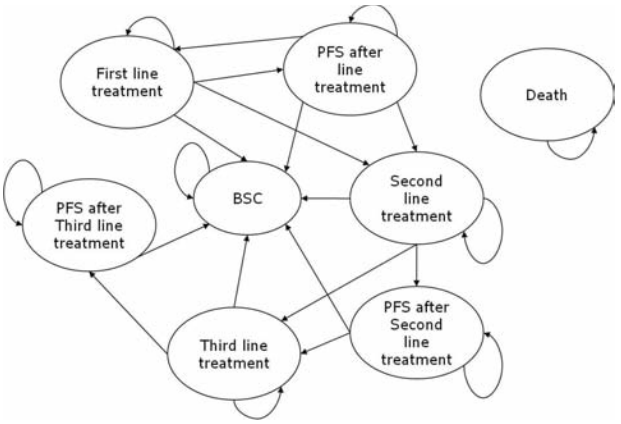

Figure 1. Structure of the Markov model

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IMPACT OF MULTIPLE MYELOMA AND ITS TREATMENT ON QUALITY OF LIFE: DEVELOPMENT OF A CONCEPTUAL MODEL

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Background. There is a paucity of published qualitative research exploring the impact of multiple myeloma (MM) and its treatment on patients' health-related quality of life (HRQL).**Aims.** To explore patient perspectives on signs/symptoms associated with MM, the burden of undergoing treatment for the disease, and the subsequent impact of these experiences on HRQL. Further, the study aimed to develop a conceptual model to illustrate the key concepts identified and the nature of their inter-relationships. **Methods.** Patients with MM were recruited into this cross-sectional qualitative study through a patient panel in the United States. An interview discussion guide was developed using information obtained from a review of published literature and through interviews with experienced MM professionals (three clinicians, one oncology nurse). The study protocol received independent ethical approval, and patients provided informed consent prior to participation. In-depth, semi-structured interviews were conducted via telephone with MM patients to explore their experiences of both the disease and its treatment. Data were analyzed using a thematic analysis approach. **Results.** To date, 15 MM patients have participated in open-ended, semi-structured interviews. The sample was diverse in terms of age (mean: 60.07 years; SD: 6.65; range: 47-72), and included 8 male and 7 female patients. The patients were at various stages of treatment (10 receiving second-line treatment or beyond; 4 receiving first-line treatment; 1 newly diagnosed who had not received any treatment). Patients reported signs/symptoms they currently or previously experienced from MM. Almost all had experienced fatigue (93.3%) and pain (86.7%); other commonly reported burdens were bone damage or fractures (40.0%), anemia (33.3%), and nausea, neuropathy, shortness of breath, aches, and infections (each 20%). Qualitative data analyses revealed that undergoing therapy for MM can have a profound impact on patients' HRQL. MM signs/symptoms were a major cause of distress, with most patients experiencing difficulties as a result of bone pain and/or severe fatigue. Interventions aimed at controlling MM signs/symptoms could themselves pose challenges for patients' HRQL. A wide range of treatment-related adverse events were reported including fatigue, neuropathy, insomnia, and gastrointestinal symptoms. Undergoing treatment for MM placed a substantial psychological and physical burden on patients; in addition, it could result in significant disruption of social and leisure activities, as well as loss of independence, and impact on relationships. A conceptual model was subsequently developed to illustrate the relationship between the different concepts and their moderating factors.**Conclusions:** The conceptual model developed in this study graphically depicts influences on HRQL for those undergoing MM therapy. To our knowledge this is the first formal attempt to create a comprehensive model of HRQL in MM that explores the inter-relationships between signs/symptoms, treatment burden and HRQL concepts. Using an open-ended qualitative approach allows important issues to be reported by patients that may not be highlighted in standard HRQL questionnaires. The model demonstrates that the determination of HRQL is complex and extends beyond either simple disease progression or treatment response. This model may prove useful in informing measurement strategies, developing new instruments, or selecting endpoints for future trials in MM.

1091

LIVING WITH MYELOMA-RELATED PAIN: PERCEPTIONS OF UK PATIENTS, CARERS AND CONSULTANTS

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Background. Pain is the most common symptom of myeloma and will affect up to 80% of patients at some point. It can have a huge impact on the patient's quality of life, particularly if it is untreated or poorly managed. For many myeloma patients, pain can be frustrating and debilitating, affecting them physically, emotionally and socially. It can also have a significant impact on their carers and family members. **Aims.** To ask patients, their carers and consultants about the impact of living with myeloma-related pain, to establish if there are differences in perceptions between those who are directly impacted by pain and those who treat and manage it. **Methods.** Patients and carers were invited to complete a questionnaire that was available as a paper copy and online. Consultant haematologists were asked to complete an online survey. Further qualitative data was collected through ten one-to-one interviews with patients and carers. The survey was devised and conducted by Myeloma UK and supported by a grant from Napp Pharmaceuticals Limited. **Results.** 87 patients, 56 carers and 10 consultants participated in the survey. 68% of patients reported that they lived with pain every day or most days. Most (76%) said that on average, their pain was mild or moderate in intensity. Patients reported that pain interfered with day-to-day activities (84% of patients), ability to work (82%), mobility (86%), sleep (74%), mood (74%), and social life/relationships (81%). 81% of carers said they thought that the patient they care for lived with pain every day or most days and 60% of consultants thought they got a better understanding of the patient's pain from the carer. Compared with patients, carers perceived that pain impacted more on mood, mobility and day-to-day activities. Most patients (85%) and carers (77%) thought that the consultant had a good understanding of the patient's pain; 80% of consultants were confident about their management of myeloma-related pain, 70% said they often initiated discussions with patients about their pain. However, 50% of consultants said that managing side-effects of pain treatment was a challenge and 40% said they didn't have enough time in clinic to accurately assess the patient's pain. Perceptions of compliance with medication differed: 10% of consultants, 90% of patients and 82% of carers thought the patient always took the pain-killers prescribed to them. **Summary and Conclusions.** The survey results confirm that pain is a common and debilitating symptom of myeloma. It is difficult to ascertain if the difference between the proportions of patients and carers reporting myeloma-related pain reflect over-reporting by carers (to protect the patient and ensure pain is treated) or under-reporting by patients (to avoid being a burden on the carer). Communication between patients, carers and consultants does not seem to be an issue, suggesting that where pain is undertreated, it is due to practical issues such as limited clinic time, lack of pain specialist support and a need for better short-acting, myeloma-specific agents.

SIMULTANEOUS SESSION II

Acute myeloid leukemia - Trial design and novel agents

1091A

EVALUATION OF THE CLINICAL IMPACT OF DIFFERENT CATEGORIES IN AML WITH MDS-RELATED CHANGES DEFINED ACCORDING TO THE WHO CLASSIFICATION IN A LARGE COHORT OF 2392 NEWLY DIAGNOSED ADULT AML PATIENTS

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Background. The current WHO classification defines acute myeloid leukemia (AML) with a prior history of myelodysplastic/myeloproliferative neoplasm (MDS/MPS), AML with multilineage dysplasia and AML with specific cytogenetic abnormalities as AML with MDS-related changes (MRC-AML). These patients are considered to have an inferior outcome compared with *de novo* AML. Whether this is due to an adverse genetic risk profile or by the fact of MRC-AML per se remains open. In addition, the prognostic impact of the different subcategories has not been evaluated. The minimal time period between diagnosis of preceding MDS/MPN and subsequent AML is defined differently (≥one month by Cheson et al. JCO 2003 versus ≥six months according to WHO 2001). **Aims.** To study the clinical impact of MRC-AML in a large cohort of newly diagnosed AML patients in the context of clinical and genetic characteristics. **Methods.** The study included 3177 adult patients (median age, 54.5 years; range, 16-85 years), entered on five protocols of the German-Austrian AML Study Group between 1993 and 2008, using intensive induction and consolidation therapy. Information on type of AML, karyotype and molecular marker status of *NPM1*, *FLT3* and *CEBPA* was available in 96%, 91%, 90%, 85% and 74% of the patients, respectively. Patients with therapy-related AML (n=200), those with recurrent cytogenetic abnormalities according to the WHO classification (n=452) and those lacking information on type of AML (n=133) were excluded. **Results.** Of 2392 patients, 210 (9%) had a history of preceding MDS/MPN (n=69, one to six months; n=141, >six months) and 197 (8%) had evidence of multilineage dysplasia. MDS-related cytogenetic abnormalities were present in 468 of 2099 (22%) patients with available cytogenetics, in 51 of 201 (25%) with prior MDS/MPN and in 56 of 197 (28%) patients with multilineage dysplasia. MRC-AML patients were significantly older compared with *de novo* AML (median age, 58 versus 53 years, p<0.0001) with highest median age in the subgroup defined by a history of preceding MDS/MPN of at least six months (median age, 62 years). White blood counts were significantly and consistently lower (p<0.0001) in MRC-AML (median 5.0/nl) compared with *de novo* AML (median, 17.4/nl), whereas percentage of blast cells in bone marrow and peripheral blood were equally high in *de novo* AML and AML with MDS-related cytogenetics, but substantially lower in all other subcategories of MRC-AML. Notably, the incidence of *NPM1*-wildtype/*FLT3*-ITDnegative/*CEBPA*negative (triple negative) normal karyotype AML was more than twice as high in MRC-AML compared with *de novo* AML (62% versus 24%; p<0.0001). In uni- as well as multivariable analyses MRC-AML was associated with an inferior outcome for all clinical endpoints compared with *de novo* AML. Within the group of MRC-AML, the subcategory defined by multilineage dysplasia was associated with a superior outcome compared with the other MRC-AML subcategories. Of note, in the genetically defined subset of triple-negative normal karyotype AML MRC-AML lost its prognostic impact. **Conclusions.** In this large cohort of adult AML patients, MRC-AML was an adverse prognostic factor. However, in genetically well defined subgroups this prognostic impact gets lost.

1091B

THE GERMAN AML INTERGROUP STUDY: COMPARISON OF OUTCOME OF THE DIFFERENT TREATMENT STRATEGIES OF FIVE STUDY GROUPS WITH A COMMON STANDARD TREATMENT WHILE ADJUSTING FOR INFLUENTIAL BASELINE VARIABLES

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Background. Patients with acute myeloid leukemia (AML) used to participate in different clinical trials launched by five German study groups. Each study group pursued its own treatment strategy with respect to dosage of chemotherapy during induction and consolidation therapy as well as to the use of allogeneic hematopoietic stem cell transplantation (HSCT) and maintenance therapy. **Aims.** To possibly identify a treatment strategy superior to the others, adjusted clinical outcome was compared between the study groups. **Methods.** The five groups agreed on upfront randomization of 10% of their patients into a common standard treatment arm. Standard induction treatment consisted of two courses of cytarabine 100 mg/m²/d for one week plus daunorubicin 60 mg/m²/d on three days. Consolidation therapy consisted of either three cycles of standard high-dose cytarabine or, based on cytogenetic risk, an allogeneic HSCT. The remaining 90% of the patients of each study group were allocated / randomized to treatments according to each study groups' own trial design. Inclusion criteria were primary AML or AML secondary to cytotoxic treatment or to myelodysplastic syndrome, diagnosed at 16-60 years. Patients with acute promyelocytic leukemia were excluded. Endpoints for the intention-to-treat comparisons of each study-internal treatment strategy with the common standard treatment were percentage of complete remission (CR) and CR with incomplete recovery (CRi) after induction therapy, overall survival (OS), relapse-free survival (RFS), and event-free survival (EFS). To adjust for variations in baseline characteristics, differences in survival probabilities were assessed through multiple Cox regression. Direct adjusted survival curves based on the Cox model were estimated. Regarding CR / CRi, adjustment for prognostic variables was performed through multiple logistic regression. **Results.** Of 3171 patients eligible for analysis, 305 were randomized to the standard treatment and 828, 373, 235, 808, and 622 to the five study group-specific treatment arms, respectively. CR/CRi rate after induction therapy was 70% (95% confidence interval (CI): 65-76%) in the common standard arm and ranged from 74% to 82% in the study group-internal treatment strategies. Also when adjusted for prognostically significant baseline variables, with 82%, one study group presented a significantly better result than the standard treatment arm. With the standard treat-

ment, the adjusted five-year OS probability was 44% [95% CI: 37-50%]. The five-year OS probabilities of the studies' internal treatment strategies ranged from 42-48%. Adjusted five-year RFS probabilities were 46% [95% CI: 38-53%] in the standard arm and 36-48% in the studies' internal treatment groups. The adjusted five-year EFS probability of the standard arm was 33% [95% CI: 27-39%] and 29-39% in the studies' internal groups. Adjusting for influential baseline variables, no significant difference with regard to OS, RFS, or EFS was observed between the standard treatment arm and any of the five internal treatment strategies. **Conclusions.** Due to the lacking differences with regard to all survival endpoints, we conclude that the overall treatment strategies of the five study groups were neither worse nor better than the concept of the standard treatment arm. However, in further detailed analysis, AML subgroups may profit from specific regimen used within the study groups.

1091C

EPIGENETIC THERAPY IS ASSOCIATED WITH SIMILAR SURVIVAL COMPARED WITH INTENSIVE CHEMOTHERAPY IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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Background. The prognosis of pts with acute myeloid leukemia (AML) aged 60 and above (AML>60) is very poor. The complete remission (CR) rates among older patients with AML treated with a standard combination of cytarabine and an anthracycline (e.g. 7+3) are 35%-60% but the early (4-8 weeks) mortality is high (20-50%), which result in median survival of 4-7 months. Epigenetic therapy (decitabine, azacitabine, histone deacetylase inhibitors) is standard in MDS. We compared the outcomes of elderly patients with AML treated with standard chemotherapy vs epigenetic therapy.**Methods**. We examined 909 pts with newly diagnosed AML>60 treated either with epigenetic therapy (n=130, 78 decitabine-based, 52 azacitidine-based) or with intensive chemotherapy (n=779) between 2000 and 2010. Of the 779 pts receiving chemotherapy, 34% received AI (ara-C 1.5g/m^{2}x3d and idarubicin 12mg/m^{2}x3d) and 22% high dose araC-based chemotherapy. No differences were observed regarding cytogenetic grouping (p=0.44), including the proportion of patients with poor karyotypes (25% vs 32%; p=0.13), or regarding performance status 0-2 (740 [95%] vs 124 [95%], p=0.8).

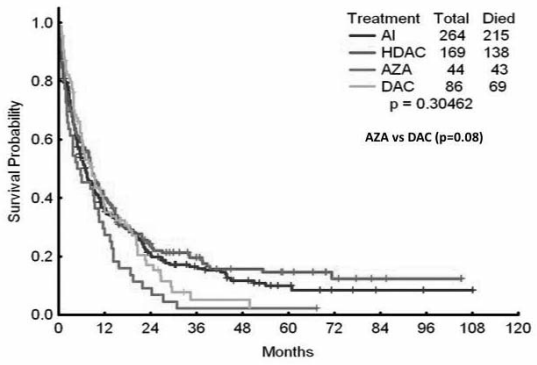

Figure 1. Overall survival of patients with AML>60 according to therapeutic modality

Results. The CR rates for patients treated with chemotherapy and epigenetic therapy were 47% and 28%, respectively (p=0.0001) while the overall response rate (ORR=CR+CR with incomplete platelet recovery) was 53% and 29% (p=0.0001). Early mortality rates (8 weeks) for both groups were 16% and 11%, respectively (p=0.13). The 2-year relapse free survival (RFS) and OS for the chemotherapy and epigenetic therapy groups were similar at 37% vs 37% mos (p=0.9) and 7.6 vs 6.9 mos (p=0.13), respectively. When the analysis was limited to the 325 pts with poor risk cytogenetics (e.g. -5 and/or -7, complex), the CR rates for pts receiving chemotherapy (n=271) or epigenetic therapy (n=54) were 31% and 33% (p=0.06) while median OS was 4.2 vs 6.2 weeks (p=0.3), respectively. The corresponding CR rates for patients with diploid karyotype were 58% vs 31% (p=0.0001). However, median OS was 12.7 vs 9.5 mos, p=0.088. Similarly, CR rates for patients carrying the *FLT3*-ITD mutation were 56% vs 42% (p=0.469) with median OS of 7.6 vs 9.2 months (p=0.73). The CR rate (25% vs 29%, p=0.6) and OS (4.8 vs 8.5 mos, p=0.08) for pts treated with azacitidine/azacitidine-based or decitabine/decitabine-based therapy showed a trend towards improved outcomes with decitabine. A multivariate analysis of these prognostic factors for survival identified the following to be independently adverse: older age (p=0.002), adverse cytogenetics (p=0.004), poor performance (p<0.0001), elevated creatinine (p<0.0001), leukocytosis (p=0.002), and prior chemotherapy for other cancers (p=0.2). Repeating the multivariate analysis for pts ≥70 years identified the same first 4 prognostic factors. The median OS of pts who failed (but not died after) initial AML therapy (114 chemotherapy, 33 azacitidine, 60 decitabine) after first salvage therapy was 1.2 mos with intensive chemotherapy, 1.1 mos with azacitidine and 3.1 mos with decitabine (p=0.036). **Conclusions**. This retrospective analysis in a large cohort of pts with AML>60 treated at our institution over the last decade showed similar long-term outcomes with epigenetic therapy versus intensive chemotherapy

1091D

THE SEQUENTIAL COMBINATION OF GEMTUZUMAB OZOGAMICIN AND INTENSIVE CHEMOTHERAPY DOES NOT BENEFIT OLDER PATIENTS WITH UNTREATED AML: RESULTS OF THE EORTC-GIMEMA AML-17 RANDOMIZED TRIAL

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Background. Gemtuzumab ozogamicin (GO), a calicheamicin-linked anti-CD33 monoclonal antibody, has clinical activity in relapsed AML and has recently been shown to significantly improve survival in older patients when administered in combination with frontline standard chemotherapy (Burnett et al, Blood 2011: a582; Castaigne et al, Blood 2011: a6). **Aims.** Study AML-17 is an open-label randomized phase III trial to evaluate the benefit and toxicity of adding upfront GO to standard induction and consolidation therapy in older patients with untreated AML. **Methods.** Eligible patients (age 61-75 years; WHO PS 0-2; maximum allowed WBC pre-therapy 30x10^9/L [a short course of HU permitted]) with newly diagnosed non-M3 AML were randomized (stratified by age, initial WBC,% CD33 expression, and Institution) to receive, or not, two upfront infusions of GO (6 mg/m^2 on day 1 and 15) prior to a course of MICE (mitoxantrone, cytarabine, etoposide), to be started within 10 days from response assessment to GO (day +43) or sooner whenever disease progression was documented. Patients achieving CR or CRp received consolidation therapy with 2 courses of ICE (idarubicin, cytarabine, etoposide) with or without a single infusion of GO (3 mg/m^2 on day -1 of each course), according to their initial assignment. The primary endpoint was OS; secondary endpoints included CR+CRp rate after induction, RFS, and toxicity. A total of 378 deaths were required in order to detect an increase in OS at 2.5 years from 20% (No GO) to 30% (GO): HR=0.75 (alpha=5%, power=80%). For efficacy endpoints, all analyses were performed according to the intent-to-treat principle. **Results.** Between 09/2002 and 08/2007, 472 patients (median age 67 years) were randomized in the study by 60 centers. After induction CR/CRp was achieved in 223 pts (47%), and at a median follow-up of 5.2 years 414 deaths were reported. As shown in the Table, induction response was comparable in the two arms, but GO treatment was associated with a higher 60-day mortality (mostly due to adverse events) and a shorter OS. Importantly, age emerged as a significant predictor of outcome: while treatment results were comparable in the younger age cohort (61-69 years), pts older than 70 years of age who received GO, as compared with those in the same age group treated with standard chemotherapy only, fared significantly worse in terms of both induction response (P=0.01) and OS (P=0.002), and had a higher 60-day mortality rate. The most common grade

3 or higher adverse events were febrile neutropenia and infection in each treatment arm. Severe liver toxicity occurred in 10% of pts during GO induction resulting in two fatalities. **Conclusions.** In this study, the sequential addition of GO to frontline intensive chemotherapy in older pts with AML was not beneficial overall, and even detrimental in those aged 70 years or more due to a higher risk of early mortality.

Table.

	All patients		Age 61-69 yr		Age 70-75 yr	
	No GO	GO	No GO	GO	No GO	GO
N. Patients	236	236	152	153	84	83
CR+CRp	116 (49%)	107 (45%)	72 (47%)	80 (52%)	44 (52%)	27 (32%)
OR (CI) p-value	0.86 (95%; 0.60-1.23) 0.46		1.22 (99%; 0.67-2.20) 0.42		0.44 (99%; 0.19-1.00) 0.01	
60d mortality (all causes)	17.8%	22.5%	15.8%	17.1%	21.4%	32.5%
2.5y OS (median, mos)	21.7% (10.0)	16.0% (7.1)	21.8% (10.1)	20.4% (8.8)	21.4% (9.1)	7.8% (4.0)
HR (CI) p-value	1.20 (95%; 0.99-1.45) 0.07		1.05 (99%; 0.76-1.45) 0.69		1.64 (99%; 1.08-2.51) 0.002	
2.5y RFS (median, mos)	18% (9.1)	17.4% (9.5)	22.1% (10.1)	17.5% (10.7)	11.4% (7.8)	18.5% (6.2)
HR (CI)	1.08 (95%; 0.81-1.44)		1.14 (99%; 0.71-1.81)		1.11 (99%; 0.57-2.20)	

1091E

F14512 A NOVEL POLYAMINE-VECTORIZED ANTI-CANCER DRUG TARGETING TOPOISOMERASE II IN ADULTS PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): RESULTS FROM A PHASE 1 STUDY

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Background. F14512 is a novel class of targeted cytotoxic agent that exploits the polyamine transport system (PTS) to deliver a drug selectively into cancer cells. This polyamine (spermine)-conjugated epipodophyllotoxin is 10-fold more potent than etoposide *in vitro*. This is most likely a consequence of an increased affinity for DNA and a stabilisation of the DNA/Topoisomerase II complex mediated by the polyamine moiety. **Aims.** This first-in man multicenter phase I study was designed to determine the maximal tolerated dose (MTD) of F14512 single agent in patients (pts) with relapsed or refractory AML by determining the incidence of dose-limiting toxicities (DLT) during the first cycle. Secondary objectives include safety, pharmacokinetics (PK) and anti-leukemic activity. **Methods.** Eligible pts were adults aged 18-75 years with *de novo* or secondary AML and previously treated with a maximum of 3 prior induction regimens. Pts received i.v. administration of F14512 from day 1 to day 5 of each cycle. Cycles were repeated every 2 to 6 weeks depending on dose levels, leukaemia response, recovery to sufficient haematopoiesis and resolution of toxicities. Dose escalation was based initially on an accelerated titration design with 50% increment and one pt by dose level until 1 DLT was observed. The activity of the PTS was assessed for each patient on blasts cells and lymphocytes at baseline and blood samples for PK analysis were taken on days 1 and 5 of cycle 1. **Results.** From January 2010 to November 2011, 39 pts (24 males) were treated in 6 participating sites through 12 dose levels ranging from 1 to 44 mg/m^2/day. Median age was 68 years (range: 22-75 years). 27 pts had *de novo* AML, 12 had secondary AML. 14 pts had unfavourable and 18 had intermediate cytogenetics. Drug related adverse events (AE) were reported in 36 pts (92 %). The most frequent grade 3-4 drug-related AEs (≥ 5%) included reversible hypomagnesemia (23%), neutropenia (18%), sepsis (13%), febrile neutropenia (10%), asthenia (5%), hypokaliemia (5%) and thrombocytopenia (5%). Three DLT were reported: second degree atrioventricular block in 1 pt at 15.2 mg/m^2/day, non-blastic aplasia during more than 6 weeks in 1 pt and early life-threatening sepsis in 1 pt at 44 mg/m^2/day. Therefore, MTD was reached at 44 mg/m^2/day, dose at which 2 pts out of 4 experienced a DLT. Anti-leukemic activity was shown at different dose levels: 4 complete responses (CR, 10%) at 1, 15.2, 34 and 39 mg/m^2/day, 3 complete responses with incomplete recovery (CRi, 8%) at 6.8, 34 and 44 mg/m^2/day. Of note, 3 pts experienced haematological improvements respectively at 10.1, 15.2 and 26 mg/m^2/day. The PTS activity results are presented separately. The preliminary PK results illustrated an increase of drug exposure proportional to the dose, and reproducible PK behaviour between days. **Conclusions.** This phase I determined the MTD of F14512 single agent to be 44 mg/m^2/day. Accrual is still ongoing at the recommended dose at 39 mg/m^2/day. Clinical outcome in this heavily pre treated population seems promising and updated results will be presented at the meeting.

Pediatric acute lymphoblastic leukemia

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ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN WITH DOWN SYNDROME: A REPORT FROM THE PONTE DI LEGNO STUDY GROUP

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Background. Children with Down syndrome (DS) have an increased risk of developing B-cell precursor acute lymphoblastic leukemia (BCP-ALL) characterized by a low frequency of the common genetic aberrations, and a high frequency of *CRLF2* and *JAK* aberrations.**Aims.** Because DS ALL is relatively rare (only 2% of ALL patients), the clinical outcome, treatment-related mortality (TRM) and prognostic factors of DS-ALL patients treated in contemporary protocols are uncertain. Hence, we performed a retrospective study within the International ALL ("Ponte di Legno") Working Group to investigate clinical outcome and to identify prognostic factors in DS ALL patients. The further aim of the study was to translate new insights of the biology of DS ALL into improved risk stratification and hence more accurate tailoring of therapy. **Methods.** We retrospectively analysed 653 DS children with BCP-ALL treated in clinical trials of 16 collaborative study groups between 1995 and 2005. A predefined set of data was collected for each patient, consisting of clinical data obtained at diagnosis, treatment, cytogenetic and molecular data. All genotypes obtained from conventional karyotyping, FISH or RT-PCR were centrally reviewed and assigned to specific cytogenetic groups. A Dutch Childhood Oncology Group (DCOG) non-DS BCP-ALL cohort (n=827) from the same treatment era served as a reference cohort.**Results.** DS ALL patients had a higher 8-year cumulative incidence of relapse (CIR) (26±2% vs. 18±1%; p=0.001) and higher 2-year TRM (7±1% vs. 1.0±<0.1%; p<0.0001) than non-DS BCP patients, resulting in lower 8-year event free survival (EFS) (64±2% vs. 78±1%; p<0.0001) and overall survival (74±2% vs. 86±1%; p<0.0001). Children age <6 years with WBC <10x109/L had the best outcome (EFS 78±3%, TRM 3±1%, CIR 17±3%), confirmed by multivariate Cox regression analyses (EFS: age < 6 years hazard ratio (HR) =0.58, p=0.002, WBC <10x109/L HR =0.58, p=0.002). The 8-year EFS of subjects with ETV6-RUNX1 (8.3%) was 95±4%, similar to non-DS ALL patients. Within high hyperdiploid (HeH, modal number >50) DS ALL, 45% of the patients had trisomies 4&10 with EFS of 88±8%, which was explained by reduced TRM rate. Neither *JAK2* R683 nor genomic *CRLF2* aberrations were associated with outcome (n= 30 and n=93 respectively). TRM was not restricted to a specific treatment-phase or treatment-regimen. Specifically, inclusion of anthracyclines in induction had no impact on TRM (1.5±1% vs. 1.7±1%; p=0.46) or EFS (64±6% vs. 69±5%; p=0.39).**Summary / Conclusion:** This large international study showed that age <6 year, WBC <10x10^9/L and the presence of *ETV6-RUNX1* or trisomies 4&10 are favourable prognostic factors within DS ALL which may be used to guide future risk-adapted treatment. DS ALL patients have a relatively poor survival compared to non-DS ALL patients, primarily due to a higher relapse rate, but also to a higher TRM. The occurrence of TRM throughout the entire treatment period and the lack of association with specific treatment elements suggest the need for improved supportive care strategies. Furthermore, there is a need for caution in treatment reduction for the majority of DS ALL patients that lack the good prognostic features, as relapse is a major problem.

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THE TIMING AND SUB-CLONAL GENETIC ARCHITECTURE OF CRLF2 REARRANGEMENTS IN DOWN'S SYNDROME ACUTE LYMPHOBLASTIC LEUKAEMIA

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Children with Down's syndrome (DS) have a 40 fold increased risk of acute leukaemia with approximately half developing acute lymphoblastic leukaemia (ALL). These leukaemias are a cytogenetically distinctive group, with a lower incidence of the common chromosomal translocations in childhood ALL. 60% of DS-ALLs show overexpression of *CRLF2* due to rearrangements including translocations, t(X;14) or t(Y;14), juxtaposing *CRLF2* to the *IGH* enhancer or deletions resulting in a *P2RY8-CRLF2* fusion-gene. *CRLF2* overexpression has been associated with activation of the JAK/STAT pathway in transduced primary B- cell progenitors. It has been proposed that *CRLF2* overexpression is an early event in the evolution of DS-ALL, with activating mutations or copy number alterations (CNAs) occurring as secondary events driving leukaemogenesis. Our aim was to determine whether *CRLF2* rearrangements are a prenatal event in the pathogenesis of DS-ALL and to investigate the sub-clonal genetic architecture at a single cell level.We have been able to collect and "backtrack"six neonatal blood spots from *P2RY8-CRLF2* positive DS-ALLs, each with a unique fusion-gene break point. Blood spot screening for the fusion-gene identified two positive and four negative cards. This suggests that *CRLF2* rearrangements can occur before birth but may not necessarily always do so (negative blood spots are uninterpretable).To define the genetic architecture of six *CRLF2* rearranged DS-ALLs (including one case which relapsed) we characterized patient specific genomic alterations using SNP6 arrays and mutation screening and interrogated mononuclear cell cytospins from each case using four-colour FISH. Two samples harbored *IGH-CRLF2* translocations and four were positive for the *P2RY8-CRLF2* fusion. Loss of *CDKN2A* or a small region at 6p22.22 were the most frequent CNAs, occurring in two cases. *IL7R*α mutations were found in two cases and the relapse sample was positive for the R683G *JAK2* mutation, but this was not found at diagnosis. FISH analysis revealed a branching subclonal genetic architecture similar to that identified in *ETV6-RUNX1* positive ALL (Anderson K et al; 2011). Moreover, in the relapse case, the major clone present at relapse could be found as a minor clone at diagnosis.As FISH is limited by the number of CNAs that can be analysed and cannot be used for simultaneous CNA and mutation analysis we have developed a novel multiplex Q-PCR approach using the BioMark HD (Fluidigm) to interrogate CNAs, fusion-genes and mutations simultaneously in a single cell. We have investigated a *P2RY8-CRLF2* positive DS-ALL case, which harbored an *IL7R*α mutation and loss of *CDKN2A* by standard approaches. Single cell analysis confirmed that the fusion-gene was present in 98% of cells and that 96% of these harbored a heterozygous *IL7Rα* mutation, 2% were homozygous and 2% carried the wildtype gene. Loss of both *CDKN2A* copies occurred in 11% of *P2RY8-CRLF2* positive cells, loss of one copy in 76% of cells but retention of *CDKN2A* was found in 9%. These data suggest a sequence of genetic events and sub-clonal structure with *CRLF2* close to or at the apex. This is compatible with *CRLF2* rearrangements being an early and possibly initiating event in DS-ALL.

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INDEPENDENT UNFAVORABLE PROGNOSIS FOR BCRABL1-LIKE AND IKZF1-DELETIONS IN CHILDHOOD PRECURSOR B-ALL

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Background. The prognosis of childhood precursor B-ALL has improved tremendously during the past decades. This improvement is largely due to risk-adapted therapies including e.g. the *BCRABL1*-translocation and *MLL*-rearrangement as high-risk factors. Recently, *IKZF1*-deletions and the *BCRABL1-like* signature were introduced as new unfavorable prognostic markers in pediatric ALL. The *BCRABL1-like* group is characterized by a high frequency of *IKZF1*-deletions, indicating that the (in)dependency of both prognostic factors needs to be established.**Aims**We aimed to determine the prognostic value of *IKZF1*-deletions and *BCRABL1-like* signatures in newly diagnosed children with precursor B-ALL who were treated according to 3 recent treatment protocols. In addition, we aimed to study the prognostic interaction/independence of *IKZF1*-deletions and *BCRABL1*-like signature.**Methods**Leukemic cell samples of pediatric precursor B-ALL patients enrolled in DCOG ALL-9, DCOG ALL-10, and COALL-97/03 were studied. *BCRABL1*-positive and *MLL*-rearranged cases, characterized by inferior outcome, were excluded. *BCRABL1-like* cases were identified by an established 110 probe sets classifier using Affymetrix U133 plus2.0 gene expression arrays (Den Boer et al, Lancet Oncology 2009). *IKZF1* deletions were detected by multiplex-ligation dependent probe amplification (MLPA) p335-assay (MRC Holland) and validated by an MLPA p202-assay (MRC Holland) or array-comparative genomic hybridization (Agilent, 180k array-CGH). Mutations in *IKZF1* were determined by genomic DNA- sequencing. The cumulative incidence of relapse (CIR) curves were calculated using death as competing event.**Results**Gene expression profiling of 447 pediatric precursor B-ALL cases revealed 78 (17.4%) *BCRABL1-like* cases. *IKZF1*-deletions were detected in 15% of precursor B-ALL cases with the highest frequency of 37% found in *BCRABL1-like* ALL (28/76). In 3 independent patient cohorts, as well as for all cohorts together, the 5-year cumulative incidence of relapse (CIR) of *BCRABL1-like* cases was 3-fold higher than of remaining precursor B-ALL cases (31% vs 10%, p=0.001 for all cohorts together). The 5-year CIR of *IKZF1*-deleted cases was 2.8-fold higher compared to *IKZF1*-wildtype cases (36% vs 13%, p<0.001 for all cohorts together). Strikingly, an *IKZF1*-deletion did not further impair clinical outcome within *BCRABL1*-like cases (p=ns) whereas it remained poor prognostic within non-*BCRABL1-like* cases (p<0.001). Vice versa, *BCRABL1-like* cases did not have a poorer outcome than remaining precursor B-ALL cases within the group of *IKZF1*-deleted cases (p=ns) whereas this signature was predictive for a poorer outcome within the *IKZF1*-wildtype group (p<0.001). The absence of additional prognostic value was not explained by inactivating mutations in *IKZF1*-wildtype cases and suggests that both factors are complementary but not mutually exclusive. Multivariate analysis stratified for treatment cohort revealed that the unfavorable outcome predicted by *IKZF1*-deletions (hazard ratio 2.9, p<0.001) and *BCRABL1-like* signature (hazard ratio 2.3, p=0.003) are independent of each other and of other prognostic markers including age and white blood cell count.**Conclusions**The *BCRABL1-like* signature and *IKZF1*-deletion are independent poor prognostic markers in children with precursor B-ALL. Co-presence of both factors does not further impair prognosis. These data imply that *BCRABL1-like* ALL and *IKZF1*-deleted ALL represent partially overlapping subsets of patients which may have different biology of disease. Therefore, both prognostic markers remain relevant for patient risk stratification.

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CLINICAL SIGNIFICANCE OF OCCULT CEREBROSPINAL FLUID INVOLVEMENT ASSESSED BY FLOW CYTOMETRY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The contemporary use of risk-directed systemic chemotherapy and CNS-directed treatment (intrathecal chemotherapy) has increased the 5-year relapse free survival (RFS) rates for childhood acute lymphoblastic leukemia (ALL) to 80% or more in some studies, with 90% 5-year overall survival (OS). The levels of residual disease in the bone marrow at discrete time points during therapy, has become an important prognostic factor in the management of these patients. Relapses continue to be the main cause of treatment failure, so new approaches are needed in order to identify children who relapse in the absence of known risk factors. **Aims**To assess the clinical significance of the levels of residual ALL cells in samples of cerebrospinal fluid (CSF) during therapy.**Methods**We studied 990 CSF samples from 108 patients with ALL, under an approved protocol. Samples were drawn at the time of diagnosis (108) and at each time of intrathecal therapy (882). The proportions of cells with the same immunophenotype as that of the ALL at diagnosis were assessed by multiparametric flow cytometry. Clinical data were collected from each patient for statistical analysis. **Results**Patients with CNS involvement at diagnosis (FCM+) showed a trend towards known negative prognostic factors: high risk group (36,6% vs 24%, p=0,15), higher WBC counts ($40x10^9$ vs $27x10^9$, p=0,09), T inmunophenotype (20% vs 6,4%, p=0,06), normal karyotype (23,4% vs 14%, p=0,1), and BCR-ABL fusion gene (6,7% vs 1,3%, p=0,18). The TEL-AML1 fusion gene was less represented among this cohort (10% vs 24,4%, p=0,07). No differences in RFS and OS were observed between FCM+ vs FCM- at diagnosis. Patients with CNS involvement during therapy showed significantly older age (7,1 years vs. 4,9 years, p=0,017), and higher frequencies of T-cell leukemia (30% vs 3,8%, p<0,001). Involvement of the CNS during therapy before relapse was detected in 63/882 samples. We found a significantly higher RFS and a trend to a lower OS in patients with FCM+ during therapy, especially in maintenance: 3-years RFS (72% vs. 98%, p<0,001) and 3-years OS (88% vs. 100%, p=0,2). Applying the same analysis to the 4 periods that compose therapy independently (i.e., induction, consolidation, reinduction, and maintenance), the detection of subclinical CNS disease by FCM during maintenance was associated with significantly lower 3-years RFS (61% vs. 94% p<0,001) and 3-years OS (88% vs. 98%, p=0,045).**Summary/conclusions**We show here that a sensitive methodology like flow cytometry can be applied for a close follow-up of the levels of ALL in CFS samples during therapy. The detection of residual leukemic cells at the CNS during the treatment period may identify a group of patients at high risk for relapse

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PROGNOSTIC SIGNIFICANCE OF FUSION GENE TRANSCRIPTS ASSESSMENT FOR MINIMAL RESIDUAL DISEASE MONITORING IN MLL-REARRANGED INFANT ACUTE LYMPHOBLASTIC LEUKEMIA WITHIN MLL-BABY TRIAL

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Background. *MLL* rearrangements are found in the vast majority of infant acute lymphoblastic leukemia (ALL) cases, so in this cohort of patients minimal residual disease (MRD) monitoring by detection of fusion gene transcripts(FGt) can be a reliable tool for prediction of outcome. In MLL-Baby protocol for infant ALL conventional chemotherapy is augmented by administration of all-trans retinoic acid(ATRA). **Aim**. To investigate the prognostic significance of MRD monitoring by detection of FGt in infants with *MLL*-rearranged ALL treated by MLL-Baby protocol. **Methods.** 39 infants (12 boys, 27 girls) with defined *MLL* rearrangements were included in the current study. Median age was 4.37 months (range 0.03-11.80). MRD detection was performed by nested RT-PCR and real-time quantitative PCR (RQ-PCR) in bone marrow samples, obtained at the time of diagnosis, on the day 15 and at the end of remission induction (time point (TP) 1 and TP2) and then after each course of ATRA administration (TP3-TP9).

Median of follow-up period was 32 months (range 5-99). **Results**. All patients were MRD-positive at TP1. Proportion of MRD-negative patients increased from 8% at TP2 to 67% at TP5. At later TPs number of MRD-negative patients remained stable. TP4 was the earliest TP when discriminative data was obtained. Event-free survival (EFS) in MRD-negative patients was higher while cumulative incidence of relapse (CIR) was lower in comparison with MRD-positive ones (0.79±0.09 vs 0.13±0.11, 0.20±0.01 vs 0.85±0.03, respectively, p=0.001 in both cases). MRD-positivity at TP5 also led to unfavorable outcome (EFS 0.14±0.12 vs 0.80±0.08, CIR 0.86±0.02 vs 0.19±0.0 p=0.006 and p=0.005, correspondingly). MRD data obtained at TP5 did not bring any advantages as compared with TP4. Among initial patients characteristics (age, sex, WBC count, immunophenotype, CNS-status, type of *MLL* partner gene) and treatment response parameters (day 8 blast cell count, bone marrow status on day 15, day 36 remission achievement, TP4 MRD-status) age less than 6 months and MRD-positivity at TP4 revealed prognostic significance on risk of relapse in the univariate analysis (p=0.001 and p=0.003, respectively). In Cox regression model only MRD-positivity was associated with poor outcome (hazard ratio 3.771, 95% confidential interval 1.033-13.674, p=0.044). MRD-positivity at TP4 remained significant parameter for high risk group patients(n=18). Based on receiver operator characteristic curves analysis of RQ-PCR TP3 data the threshold level for the best discrimination between patients with various outcomes was defined as 0.1%. 7 patients with MRD higher than 0.1% at TP3 had lower EFS and higher CIR compared to 21 patients with MRD at TP3 lower than 0.1% (p=0,011 and p=0,017, relatively). In the multivariate analysis MRD value at TP3 higher than 0.1% was the single independent prognostic factor(hazard ratio 4.250, 95% confidential interval 1.159-15.585, p=0.029). Comparison between presence of MRD-positivity at TP4 and MRD level higher than 0.1% at TP3 did not allow us to determine the best discriminative factor (p=0.099 and p=0.332, correspondingly). **Conclusions**. In our series level of MRD higher than 0.1% at TP3 and any MRD-positivity at TP4 were associated with unfavorable outcome. MRD data obtained at later TPs did not bring extra advantages.

Chronic lymphocytic leukemia - Biology

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EPIGENETIC UPREGULATION OF LNCRNAS AT 13Q14.3 IN LEUKEMIA CORRELATES WITH DOWNREGULATION IN CIS OF A GENE CLUSTER REGULATING NF-KB

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Background. NcRNA genes are central players in the regulation of target gene function. The mode of action varies from posttranscriptional regulation (i.e. miRNA genes) to regulation *in cis* or *in trans,* either via competition or blockage (e.g. lincRNA genes), by acting as organization complexes or as targeting factors of chromatin modifying factors (e.g. *HOTAIR, KCNLQT1* and *XIST*). NcRNA genes can even act as enhancers themselves. However, for the vast majority of ncRNAs, the cellular function remains enigmatic. In chromosomal band 13q14.3 near the retinoblastoma gene (*RB1*), two miRNA genes *miR15a* and *miR16-1* and two long non-coding RNA (lncRNA) genes *DLEU1* and *DLEU2* have been identified.AimsTo characterized the epigenetic tumor suppressor mechanism in 13q14.3 and its role in the pathogenesis of CLL. **Methods.** We used aPRIMES, MCIp (n=25 CLL, n=7 B-cells from healthy donors), bisulfite sequencing (CLL, n=7; sorted B-cells from healthy donors n=4), ChIP of macroH2A and H3K4me2 (CLL, n=7; and PBMC from healthy donors, n=5) and BioCOBRA/MassARRAY (CLL, n=82; sorted B-cells from healthy probands, n=19) to characterize the epigenetic makeup of the critical region in 13q14.3. Gene expression analysis of candidate genes in the critical region was performed by qRT-PCR in patients with normal karyotype (n=34) and sorted B-cells from healthy donors (n=20).

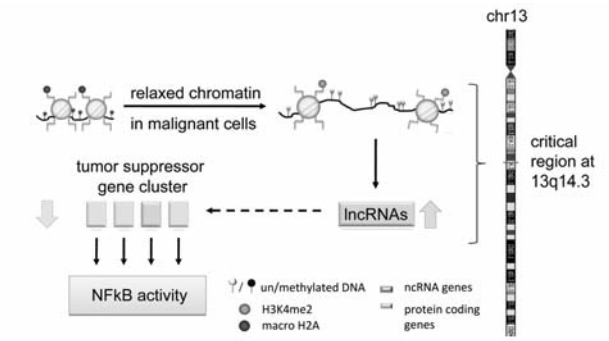

Figure 1. Model of the tumorsuppressormechanism in 13q14.3

The functional impact on the expression of candidate genes was adressed by modulation of DNA-methylation in cell lines (n=16) and luciferase reporter assays. In order to assess the functional role of the 13q14.3 candidate genes, we performed a screen of the human miRome (n=810 miR-mimics) and overexpressed and knocked down 13q14.3 candidate genes in timecourse experiments in cell lines and in primary CLL cells and quantitated the effect on NFkB signaling. Results.The critical region displays a relaxed chromatin status in CLL cells: It is enriched for activating histone modifications. The transcriptional start sites of the lncRNA genes are DNA-hypomethylated in 95% of CLL samples and is independent of cytogenetic aberrations, IGHV status and overall survival. Intriguingly, it correlates with upregulation of the lncRNA transcripts and downregulation of the protein-coding genes. This points to a regulation *in cis* of the candidate tumor suppressor genes by the lncRNA genes. 13q14.3 genes are also functionally related. *RFP2, DLEU7, KPNA3* and *KCNRG* modulate NF-kB signaling, both in cell lines and *RFP2* also in primary leukemia cells. Kinetics of NFkB induction exclude an indirect effect. Induction of NFkB by *RFP2* can be abrogated by mutation of the ubiquitin ligase activity of RFP2, coexpression of dominant negative IkB or IKK or knockdown of RelA. This points towards involvement of the canonical NFkB pathway. Moreover, a miRome-wide screen showed the *miR15/16* family localized at 13q14.3 to be the strongest inducers of NF-kB. **Conclusions.** In summary, the tumor suppressor mechanism in 13q14.3 involves long non-coding RNAgenes that are transcriptionally deregulated by DNA-demethylation in leukemic cells and arepart of a colocalized cluster of functionally related genes that modulate NF-kB signaling.

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TCL1A AND ATM ARE COEXPRESSED IN CLL

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Background. Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of B cells that are resistant to apoptosis. This resistance is induced by pro-survival stimuli of the microenvironment. *TCL1* and *ATM* are central to the pathogenesis of CLL and associated with more aggressive disease. Their protein products have recently been shown to physically interact in CLL cells and to impact on NF-kB signaling, which is a key regulator of apoptosis. **Aims.** To characterize the functional interrelation of *TCL1A* and *ATM* in CLL.MethodsWe used methyl-immunoprecipitation (MCIp) of CLL patient samples (n=13) and sorted B-cells from healthy probands (n=6) to assess DNA-methylation of *TCL1A* and *ATM* promoters in primary CLL cells. To quantitate expression of *TCL1A* and *ATM* upon complex external stimuli, CLL cells from n=6 patients were cocultured on human stromal cells (HS-5), conditioned medium from HS-5 cells and on murine stromal fibroblasts. Results from gene expression profiling of these cells at 7 different timepoints and quantitative RT-PCR were modeled for Bayesian networks of transcriptional interdependencies of candidate genes. Finally, in order to assess its role in the pathomechanism of CLL, *TCL1A* gene expression was knocked down in primary CLL cells and the impact on apoptosis was measured by FACS and staining with 7AAD, Annexin V/PI and CD19 APC. **Results.** In peripheral CD19 positive B-cells from healthy donors (n=9) and CLL patients (n=15), *TCL1A* and *ATM* show a significant coregulation (r=0.82, p=0.00005). Expression levels of *TCL1A* are partially deregulated in CLL cells by aberrant DNA methylation at the centromeric end of the promoter-associated CgP island (r=-0.42, p=0.037), while no aberrant DNA-methylation could be detected at the ATM promoter. *TCL1A* and *ATM* have recently shown to synergize in the activation of anti-apoptotic NFkB signaling. We therefore tested whether the observed coregulation of *ATM* and *TCL1A* also occurs upon complex external stimuli, e.g. by microenvironmantal anti-apoptotic support. To this end we cocultured primary CLL cells with different stimuli mimicking the microenvironmental support. Intriguingly, all external stimuli induced similar *TCL1* and *ATM* time-course kinetics. This was supported by Bayesian modeling using Markov-chains of the 8 most deregulated genes that included *TCL1A* and *ATM,* and these two genes showed the most significant coregulation. In line with a coordinative regulation of NF-kB signaling by *TCL1*, its knockdown induced apoptosis in primary CLL cells (p<0.001). These findings suggest that both genes functionally cooperate and modulate similar cellular pathways such as NF-kB. **Conclusions**. In the present study we show that *TCL1* and *ATM* are significantly coexpressed in malignant as well as non malignant B cells upon different microenvironmental stimuli, underlining their functional cooperation in the pathomechanism of CLL.

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IDENTIFICATION OF AN EXPANDED POPULATION OF TERMINALLY DIFFERENTIATED CD8+ T CELLS WITH A NOVEL PHENOTYPIC AND FUNCTIONAL PROFILE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukemia (CLL) is associated with a profound defect in T-cell function. We have previously demonstrated global alterations in gene expression and functional defects in T cells from CLL patients, which had similarities with those described in exhausted T cells seen after persistent antigenic stimulation in chronic viral infections. **Methods.** We used multiparameter flow cytometry and functional assays to determine if peripheral blood T cell populations from previously untreated CLL patients exhibited features of exhaustion. As both advanced patient age and expansion of CMV-specific T cells in CMV seropositive patients were potential confounding factors, we matched CLL patients (n=32) and healthy controls (n=12) for both age and CMV-serostatus. **Results**. We found increased expression of CD279 (PD-1), CD160, and CD244 on both CLL CD8+ and CD4+ T cell populations from CLL when compared with healthy donor T cells, *independent* of CMV-serostatus. CD160 and CD244 were co-expressed, and were preferentially upregulated on T cells lacking expression of CD28. The expression of these molecules was dependent on the stage of T-cell development, with the highest expression on the terminally differentiated T_{EMRA} subset. Of note, we did not observe CLL-induced downregulation of the IL-7 receptor (IL-7R), which is usually seen on exhausted T-cells. We identified a subset of CD8+ T cells expressing PD-1 with high intranuclear staining of Blimp-1, a transcriptional repressor implicated in T-cell exhaustion. We examined cytokine production, proliferative capacity, and cytotoxicity to characterize T cell function. In contrast to what has been described in many chronic viral infections, T cells from CLL patients showed *increased* production of interferon-γ, and normal production of IL-2, on a background. of increased expression of the Th1/Tc1 transcription factor T-bet. Compared with T cells from healthy donors, CLL T cells showed a proliferative defect, and while they retained the ability to degranulate, they exhibited severely impaired ability to lyse idiotype-pulsed dendritic cells. Confocal microscopy identified failure of granzyme B trafficking to the immunological synapse, reflecting profound cytoskeletal dysfunction. Of clinical relevance, treatment of CLL T cells with the immunomodulatory agent lenalidomide repaired granzyme localization and enhanced lytic synapse function. **Conclusions**. We have identified a novel phenotype of expanded terminally differentiated CD8+ T cells in patients with CLL, independent of CMV serostatus. While these cells show phenotypic characteristics consistent with chronic antigen stimulation and some similarities to “exhausted”T cells, they show *increased* production of interferon-γ, and do not downregulate IL-7R. This phenotypic and functional profile closely resembles lymphocytes found in the healthy intestinal epithelium, a site of chronic antigenic stimulation, which occur at low frequencies in the peripheral blood of normal donors. These T-cells exhibit significant cytotoxic activity in healthy individuals, but CD8+ T cells from CLL patients have defective cytolytic activity due to tumor cell-induced cytoskeletal defects. These observations suggest that the expansion of CD8+ T cells seen in CLL results from an aberrant response to chronic antigen stimulation. Furthermore, the defective cytolytic activity exhibited by these cells represents a potential therapeutic target.

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APRIL IS PRESENT IN THE MICROENVIONMENT OF CLL, AND AFFECTS DISEASE PROGRESSION IN THE TCL-1 TRANSGENIC MOUSE MODEL

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The tumor microenvironment is postulated as the prime source for proliferation and survival of chronic lymphocytic leukemia (CLL) cells. Although many different signals can protect CLL cells in vitro, direct evidence for lymph node (LN)-specific pro-survival signals in vivo is sparse. Recent in vitro studies point to TNF-receptor ligand family members BAFF and APRIL mediated signaling as key events in CLL survival through activation of NF-κB. CLL patients have increased serum levels of APRIL which correlates with prognosis. Despite these correlative studies, a clear role for APRIL in CLL leukemogenesis and progression is lacking. Moreover, formal proof of APRIL expression within the LN microenvironment is absent.In this study we address the role of APRIL in CLL leukemogenesis and progression in the EμTcl-1-transgenic (Tg) mouse model.Measurement of expression levels of the APRIL receptors TACI and BCMA revealed high expression levels of TACI on $CD5^+CD19^+B220^{dull}$ aged Tcl-1 derived leukemia cells as compared to residual normal B-cells, indicating susceptibility of these cells to APRIL-induced signaling. Indeed, ex vivo culturing of Tcl-1 derived leukemia cells with rhAPRIL-conditioned medium resulted in significant increased survival as compared to inactive APRIL-mutant medium.Next, we crossed Tcl-1-Tg mice with mice overexpressing APRIL (lck-hAPRIL transgenic (tg) mice) and measured development of leukemia over time. Already at 4 months of age both a relative and an absolute increase in $CD5^+CD19^+B220^{dull}$ cell population was observed in double-Tg mice as compared to both single-Tg strains. In contrast, increased relative and absolute leukemia cells Tcl-1-Tg mice started to occur at 8 months of age. Moreover, at 4 months of age double-Tg mice showed enhanced leukemia infiltration in bone marrow, spleen and peritoneal cavity. The average lifespan of double-Tg animals was only 303 days differing significant (p=0,0011) from 408 days in the single-Tg group.Although proliferation was enhanced in leukemia cells of both double-Tg and Tcl-1-Tg mice as compared to normal B cells, no clear difference was observed between the 2 strains. In contrast, apoptosis levels in spleen fragments of APRILxTcl-1 Tg mice appeared lower than that of Tcl-1-Tg mice. In order to study whether APRIL affected leukemogenesis or progression we compared development of clonality in the CDR3 region of sorted leukemia cells. In both mouse strains sorted $CD5^+CD3^-$ revealed to be oligoclonal at 4 months of age. This indicates that although Tcl-1 induced leukemogenesis develops early in life, it remains in an ‘MBL-like’ state in single-Tg mice while presence of APRIL results in rapid outgrowth of clonal cells. This was proven by adoptive transfer studies showing enhanced development of leukemia of transferred Tcl-1-tg splenocytes in APRIL-tg versus wild-type recipient mice. In vivo relevance of APRIL was corroborated by immunohistochemistry on humanCLL LN biopsies, which showed large APRIL positive cells throughout leukemic infiltrated

lymph nodes.This study shows that APRIL, which is present in the human CLL LN microenvironment, significantly enhances leukemia development in a mouse model. These findings suggest a rationale for pharmaceutical targeting of this pathway in CLL.

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THE EXPRESSION AND PHOSPHORYLATION OF HS1 ARE FINELY TUNED IN CLL PATIENTS

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Background. We previously demonstrated *i)* that HS1 expression has a profound effect on the development and progression of murine CLL, *ii)* that both leukemic cells lacking HS1 and leukemic cells carrying hyper-phosphorylated HS1 have a preferential homing to the bone marrow (BM) suggesting that the hyper-phosphorylation of HS1 leads to its inactivation rather that activation, and *iii)* that the phosphorylation status of HS1 is a potential prognostic marker in CLL, the hyper-phosphorylated form being an adverse prognostic factor.**Aims.** Our aim was twofold. First to elucidate the sites of HS1 phosphorylation and next to analyze the expression of HS1 in different tissue compartments. **Methods.** .We utilized both human and mouse samples. Primary human CLL cells were used to perform WB analyses and to identify HS1 phosphorylation sites by mass spectrometry. CLL cells were isolated from the BM and peripheral blood (PB) of the same patients and HS1 expression was comparatively analyzed. Finally we sorted different B cell populations from different lymphoid compartments in 8-week old C57BL6 mice to analyze HS1 expression.**Results.** We found that in CLL cells HS1 is differentially phosphorylated at Tyr397, where HS1 gets rapidly phosphorylated by Syk and Lyn kinases following B cell receptor stimulation. Tyr397 is not the only site of phosphorylation in hyperphosphorylated CLL cells. Preliminary data show that the total amount of Serine and Threonine is also different among different patients and we found new phosphorylation sites of HS1 in Serine and Threonine.We also investigated the expression pattern of HS1 in BM and PB of CLL patients either lacking or carrying the hyperphosphorylated form of the molecule. We observed that in the same patient HS1 is differentially expressed in the BM as compared to the PB; specifically that BM CLL cells lack HS1 expression when the protein is hyperphosphorylated in PB cells. These data suggest that HS1expression is tuned in different compartments and that cells that preferentially accumulate in the BM are the ones that have switched off the protein expression.We previously demonstrated that HS1 phosphorylation in modulated also in human normal B cells depending on the activation status of the cell. We asked whether this can occur also for HS1 expression: to this aim we analyzed the expression of HS1 in murine B cells. We isolated cells from spleen, peritoneal cavity and BM of wild type mice (C57BL6) and we sorted the distinct B cells populations based on differential surface marker expression. We observed that HS1 is differentially expressed in the lymphoid compartments, especially in the BM were HS1 is expressed at very low level in Pre and Pro-B cell lineages while it is expressed at a higher level in mature B cells.**Conclusions.** These findings strengthen our previous observation that HS1 phosphorylation has an important role in CLL. The fact that HS1 expression appears to be finely modulated in CLL patients suggests its functional role in the behaviour of leukemic cells. In addition the modulation of HS1 in normal B cells can help understanding its function in B-cell ontogeny

Chronic myeloid leukemia - Biology and prediction of response

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REGULATION OF CHRONIC MYELOID LEUKEMIA STEM CELLS BY LEUKEMIA ONCOGENE EVI1

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Background. Chronic myeloid leukemia (CML) is a hematopoietic stem cell (HSC) disease caused by BCR-ABL oncogene, and now newly targeted therapies toward CML stem cells are warranted because of the minimal effect of BCR-ABL-targeted tyrosine kinase inhibitors. Due to its similarity to HSCs, CML stem cells is worth being analyzed with a guide of HSC-specific factors, one of which is Evi1, a transcription factor highly expressed in HSCs and activated in myeloid malignancies including CML blast crisis (CML-BC). Our newly generated Evi1-GFP knock-in mice, the Evi1-reporter mouse line, proved to be HSC-reporter mice, and enabled us to dig into behavior monitoring of Evi1-high leukemia cells in vivo. **Methods.** We developed murine Evi1-reporter models of CML in a chronic phase (CML-CP) and CML-BC, the former by intercrossing Evi1-GFP knock-in mice with p210 BCR/ABL transgenic mice or retroviral induction of BCR/ABL into Evi1-GFP knock-in bone marrow cells, the latter by retroviral induction of BCR/ABL and NUP98/HOXA9. We also performed quantitative RT-PCR to evaluate EVI1 expression in the stem/progenitor fraction of bone marrow cells of healthy donors and CML-CP patients. **Results.** In CML-CP model, we found that Evi1 positive CML cells as a minor population, about 0.1-0.5% of total bone marrow cells, predominantly reside in the CML stem cell fraction (Lin- Sca-1+ c-kit+ (LSK)), but Evi1 expression is sharply downregulated even in myeloid progenitor (Lin- Sca1- c-kit+ (MP)) cells or more differentiated cells. Evi1-high CML LSKs showed an enhanced colony-forming capacity and were more quiescent than the other CML cells, which was correlated with its gene expression profile. The characteristic features of Evi1-high CML LSKs by GSEA analysis were hyper metabolism, activated TGF-beta signaling and activation of ABC transporters. Strikingly, Evi1-high CML LSKs were more resistant to in vivo nilotinib administration than Evi1-low CML LSKs. In concert with our data of Evi1-trafficking CML mouse, in CML patients, we have also recently found that CD34+ 38- CML stem cells showed higher EVI1 expression than CD34+ 38+ CML progenitor cells or total CML cells, which implies that EVI1 could mark CML stem cells as well as normal HSCs. In CML-BC model, which more differentiated MPs have a high leukemia initiating potential, a sizable fraction of MPs show distinct Evi1 expression. Evi1-high MP cells showed a colony-replating ability with active cycling status, and remarkably, in vivo transplantation assay revealed CML-BC stem cells that can recapitulate the disease are exclusively enriched in Evi1-high MPs. **Conclusions**. Our data of CML-CP model showed that high Evi1 expression could enrich CML stem cells and mark nilotinib-resistant cells, and those of CML-BC model were the first report to show that a limited fraction with high Evi1 expression within stem/progenitor cells possesses enhanced proliferative and leukemia-initiating capacities. The Evi1-trafficking leukemia model provides us with a new tool for dissecting pathogenesis and exploiting novel targeted therapies to eradicate CML stem cells, and would ensure an establishment of Evi1-related therapy for CML stem cells, which could be applied to EVI1-high malignancies.

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HIGH SENSITIVITY MUTATION SCREENING AND CLONAL ANALYSIS ALLOWED BY ULTRA-DEEP AMPLICON SEQUENCING UNCOVER THE COMPLEXITY OF BCR-ABL MUTATION STATUS IN PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS

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Background and Aims. point mutations within the Bcr-Abl kinase domain (KD) have been implicated in resistance to tyrosine kinase inhibitors (TKI) in chronic myeloid leukemia (CML) and Ph+ acute lymphoblastic leukemia (ALL) patients. Sanger sequencing (SS) is the recommended method for mutation screening, but it cannot identify mutant subpopulations <20%, nor can it discriminate polyclonal from compound mutations, unless it is preceded by cloning. The majority of patients positive by SS have a single mutant clone detectable, more rarely two or more; multiple mutations are thought to accumulate mainly after multiple lines of therapy. The recent development of ultra-deep amplicon sequencing protocols based on the Roche 454 next-generation-sequencing (NGS) technology has opened the way to a more accurate qualitative and quantitative characterization of the mutant clones that survive TKI therapy. **Methods.** longitudinal analysis of 92 samples collected at regular timepoints during treatment was retrospectively performed in 25 patients with TKI-resistant and 5 patients with TKI-sensitive CML or Ph+ ALL. NGS allowed to screen the Bcr-Abl KD with a lower detection limit of 0.1% and to reconstruct the clonal architecture of the mutated subpopulations, following quantitatively their evolution over time. SS was performed in parallel. **Results.** NGS revealed an unexpected complexity in the Bcr-Abl mutation status. In 94% of the samples, the higher sensitivity allowed to identify a variety of minor subclones (n=2-12 per sample; abundance,1%-18.8%) harbouring point mutations and more rarely insertions or deletions, alone or in addition to the dominant mutations detectable by SS. In all the cases, complexity was found to be high since diagnosis. The analysis of patients who later showed evidence of a single TKI-resistant mutation by SS (n=12) showed that, in all cases, additional low-level mutations were present - either in a small subfraction of the dominant mutated clone, or in an independent one, or both. The analysis of patients who accumulated multiple TKI-resistant mutations as assessed by SS showed that the newly acquired mutations could arise in the pre-existing mutated subclone(s) (2/13 patients), or in a previously wild-type subclone (2/13 patients), but they more often arose in parallel in both wild-type and mutated subclones (9/13 patients) - generating a complex jigsaw of multiple, competing populations harbouring different combinations of mutations. However, quantitative follow-up of these subclones showed that only one or a few take over, and some specific compound mutants (T315I+F359V; F317L+M351T..) were found to have greater 'fitness' over individual mutants, while others (T315I+E355G; Y253H+E255V..) have lower. **Conclusions.** mutation(s) detectable by SS are often the 'tip of the iceberg'. A mosaic of small mutated subclones are present since diagnosis. Depending on their absolute and relative 'fitness', some may survive TKI therapy, although only one or a few will be capable to achieve dominance; acquisition of additional mutations dictates further dynamics of shrinkage/expansion. The level of heterogeneity is reduced only transiently when a highly-resistant subclone takes over. Reasoning on the basis of mutation(s) detectable by SS may not always be sufficient to predict responsiveness to a TKI or substantiate clinical decisions.
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PACE: A PIVOTAL PHASE 2 TRIAL OF PONATINIB IN PATIENTS WITH CML AND PH+ALL RESISTANT OR INTOLERANT TO DASATINIB OR NILOTINIB, OR WITH THE T315I MUTATION

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Background. Ponatinib is a potent, oral, pan-BCR-ABL inhibitor active against the native enzyme and all tested resistant mutants, including the uniformly resistant T315I mutation. **Methods.** The PACE (Ponatinib Ph+ALL and CML Evaluation) trial started in Sept 2010. Patients with refractory CML (CP, AP or BP) or Ph+ALL resistant or intolerant (R/I) to dasatinib or nilotinib or with T315I received 45 mg ponatinib orally once daily. The trial is ongoing; enrollment completed in Sept 2011. Data as of 17 Jan 2012 are reported. Median follow-up was 6.6 months. **Results**. In total, 449 patients were enrolled, 5 of whom were ineligible (post-imatinib, non-T315I) but treated. Median age was 59 (18-94) years; 53% were male. Diagnoses were: 271 CP-CML (R/I=207; T315I=64); 79 AP-CML (R*60; T315I=19); 94 BP/ALL (*48; T315I=46). Median time from diagnosis to ponatinib was 6 years. Prior TKIs included imatinib (96%), dasatinib (85%), nilotinib (66%), bosutinib (7%); 94% failed >2 prior TKIs, 59% failed >3 prior TKIs. A total of 83% had a history of resistance to dasatinib or nilotinib; 12% were purely intolerant. In CP, best response to most recent dasatinib or nilotinib was MCyR 25%. Frequent mutations confirmed at entry included 29% T315I, 8% F317L, 4% E255K, 4 % F359V, and 3% G250E. Response rates are presented in Table 1. Overall, 64% remained on therapy (77% CP) at the time of reporting. Most frequent reasons for discontinuation were progression (12%) and AE (10%). Most common drug- related AEs were thrombocytopenia (33%), rash (33%), and dry skin (26%). **Conclusions**. Ponatinib has substantial activity in heavily pretreated patients and those with refractory T315I. Response rates continue to improve with longer follow-up. Multivariate analyses of predictors of outcome will be presented.

TABLE 1	n Response to Ponatinib / N Evaluable (%)		
	Overall	R/I	T315I
CP-CML			
MCyR*	126/258 (49)	88/197 (45)	38/61 (62)
CCyR	105/258 (41)	70/197 (36)	35/61 (57)
MMR	68/265 (26)	40/205 (20)	28/60 (47)
AP-CML			
MHR*	38/57 (67)	31/43 (72)	7/14 (50)
MCyR	27/72 (38)	18/55 (33)	9/17 (53)
CCyR	12/72 (17)	8/55 (15)	4/17 (24)
BP-CML/Ph+ALL			
MHR*	33/89 (37)	17/46 (37)	16/43 (37)
MCyR	30/82 (37)	14/41 (34)	16/41 (39)
CCyR	23/82 (28)	11/41 (27)	12/41 (29)
*Primary endpoints: MCyR in CP; MHR in AP, BP/Ph+ALL (baseline MHR excluded)			

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THE EUTOS HIGH RISK POPULATION DIFFERS SUBSTANTIALLY FROM THE EURO HIGH RISK GROUP; THE EUTOS SCORE PREDICTS MOLECULAR RESPONSE IN CHRONIC PHASE CML PATIENTS: RESULTS OF THE GERMAN CML-STUDY IV

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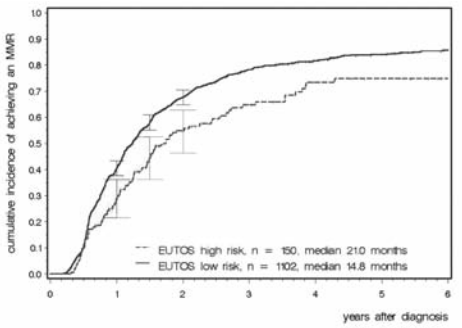
Background. The EUTOS Score was validated as a prognostic tool for the achievement of complete cytogenetic response at 18 months for chronic-phase (CP) CML patients under imatinib therapy. Using only two variables at diagnosis (basophils and spleen size), the score identifies high-risk patients with a better positive predictive value than the Sokal and EURO scores (Hasford et al. Blood 2011). **Aims**. We sought to evaluate the clinical impact of the EUTOS score to predict molecular response and to analyze the composition of the risk groups compared to the EURO score. We analyzed patients from the German CML-Study IV, a randomized 5-arm trial (imatinib 400 mg vs. imatinib 800 mg vs. imatinib in combination with interferon-alpha vs. imatinib in combination with araC vs. imatinib after interferon failure). **Results**. From July 2002 to December 2011, 1,541 patients with CP CML were randomized. 1,337 patients were evaluable for overall and progression-free survival (OS and PFS), 1,252 for molecular responses. 749 of these patients were part of the score development project. Therefore cytogenetic analyses were not described here. According to the EURO score, 36% of patients (n=475) were classified as low-risk, 51% (n=681) as intermediate-risk, and 12% (n=167) as high-risk. Applying the EUTOS score, 88% belonged to low-risk (n=1163) and 12% to the high-risk group (n=160). 12% of the high-risk patients using EUTOS score would have been classified as low-risk (n=19) according to EURO score, 45% as intermediate- (n=68) and in 43% as high-risk (n=73). Vice versa, 94 high-risk patients according to EURO (56%) were classified as low-risk using EUTOS score. Patients with high-, intermediate-, and low-risk EURO score achieved MMR after a median of 22, 16, and 13 months (p-values: 0.03 for low- vs. intermediate-risk groups and <0.001 for high- vs. low/intermediate-risk groups) and MR[4] (BCR-ABL <=0.01%) in 59, 41, and 34 months (p-values: 0.04 for low- vs. intermediate-risk groups and <0.001 for high- vs. low/intermediate-risk groups). At 12 months, the proportion of patients in MMR was 38%, 46%, 54% for high-, intermediate-, and low-risk patients, respectively. Patients with high-risk EUTOS score achieved molecular responses (MMR and MR[4]) significantly later than patients with low-risk EUTOS score (time to MMR: median 21.0 vs. 14.8 months, p<0.001, Fig. 1; time to MR[4]: median 60.6 vs. 37.2 months, p<0.001). Significantly less EUTOS high-risk patients achieved MMR at 12 months than EUTOS low-risk patients (31% vs. 51%, p<0.001). OS after 5 years was 85% for high- and 91% for low-risk patients (p=n.s.), PFS was 85% and 90%, respectively (p=n.s.). **Conclusions**. The EUTOS high-risk population differs substantially from the EURO high-risk population. Therefore the results of clinical trials using different scores have to be interpreted with utmost caution. The EUTOS score clearly separates CML patients according to MMR and MR[4] and should be used in future trials with tyrosine kinase inhibitors in CML.



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AN EXPLORATORY ANALYSIS FROM 3-YEAR DASISION FOLLOW-UP EXAMINING THE IMPACT ON PATIENT OUTCOMES OF EARLY COMPLETE CYTOGENETIC RESPONSE AT 3 MONTHS AND MAJOR MOLECULAR RESPONSE AT 12 MONTHS

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Background. In the phase 3 DASISION trial of dasatinib v imatinib in patients with newly diagnosed chronic myeloid leukemia-chronic phase (CML-CP), dasatinib showed superior efficacy over imatinib with higher rates of complete cytogenetic response (CCyR) and major molecular response (MMR) at 12 months, lower rates of transformation and acceptable tolerability (Kantarjian et al. Blood 2012;119:1123). The value of earlier and deeper response in outcome prediction has emerged in CML-CP patients receiving first-line imatinib. A recent analysis of patients treated with dasatinib or nilotinib as initial therapy for CML has shown that patients who achieve a CCyR at 3 months have significantly higher probability of event-free survival at 3 years than those without CCyR (Jabbour et al. JCO 2011;29:4260). **Aims.** Explore landmark analyses of CCyR at 3 months and MMR at 12 months on PFS. **Methods.** Patients with CML-CP diagnosed within 3 months were randomized to receive dasatinib 100 mg QD (n=259) or 400 mg imatinib QD (n=260). MMR was defined as a BCR-ABL/ABL transcript level of ≤ 0.1% on the international scale (3-log reduction compared to the standardized baseline). PFS events considered were increasing WBC; loss of complete hematologic response or major cytogenetic response; progression to AP/BP; or death from all causes. Kaplan Meier curves of progression in patients achieving or not achieving CCyR at 3 months were generated. **Results.** Patients receiving dasatinib v imatinib had faster responses; median time to CCyR and MMR was 3.2 v 6.0 months and 15 v 36 months, respectively. Higher rates of CCyR at 3 months were achieved in patients receiving dasatinib v imatinib (54% v 31%). The rates of MMR at 12 months were also higher for patients receiving dasatinib v imatinib (47% v 28%). Of those patients who achieved MMR at 12 months, 97% and 92% of patients receiving dasatinib v imatinib, respectively, maintained their MMR at 24 months. In a landmark analysis, CCyR at 3 months was associated with higher PFS rates (p-value 0.0372). Similarly, achieving an MMR at 12 months was also associated with higher PFS (p-value 0.0380). Given the low number of events and exploratory nature of this analysis these data should be interpreted cautiously. Additional analyses will be reported exploring measures potentially predictive of improved outcome. Updated analyses will be presented with minimum 36-month follow-up. **Conclusions.** In patients with newly diagnosed CML-CP, first-line dasatinib resulted in faster and deeper responses compared with imatinib. The achievement of an early deep response at 3 and 12 months appear predictive of improved PFS in patients with early CML-CP treated with tyrosine kinase inhibitors.

Hodgkin's lymphoma

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THE MICROENVIRONMENT IN CLASSICAL HODGKIN LYMPHOMA SHOWS EVIDENCE OF A TH1 AND NOT TH2 RESPONSE WITH TBET EXPRESSION BEING ASSOCIATED WITH IMPROVED SURVIVAL AND EBV STATUS

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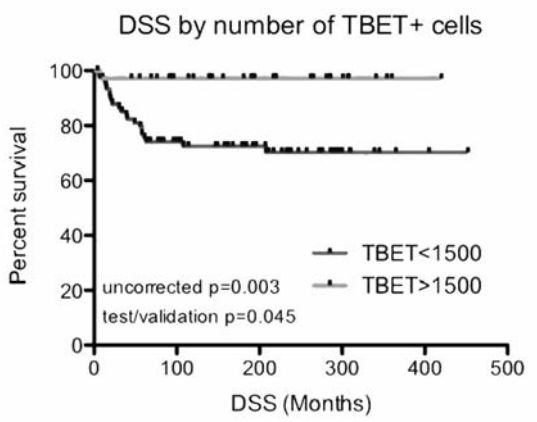
Background. The microenvironment in classical Hodgkin lymphoma (CHL) is dominated by CD4+ T cells with EBV detected in 20-30% of cases. The microenvironment has been described as TH2-dominated and suppressive although functional evidence is sparse. High FOXP3/Treg infiltration is associated with favourable prognosis but the mechanism for this is uncertain. The significance of TH1 cells remains unclear although some literature suggests this may be associated with EBV. **Aims.** Using tissue microarray (TMA) immunohistochemistry with automated image analysis we assessed expression of transcription factors (TF) including TBET (TH1) and CMAF/GATA3 (TH2), validated with single-cell cytokine profiling of frozen single-cell-suspensions (SCSs) from patient-derived tissue and determined prognostic associations. **Methods.** Formalin-fixed paraffin-embedded tissue was available for 110 patients: median age 30, 65% male, 71% advanced stage, median follow-up 16.5 years (2-40). Triplicate TMA cores were stained for TBET, GATA3, CMAF and EBER-in-situ-hybridization. Cells were counted using an automated system, expressed as total numbers per 1mm^2 and patient groups discriminated using cutpoints based on numbers of cells expressing each marker using a test/validation set methodology. Survival was analysed using the Kaplan-Meier method for overall survival (OS), disease-specific survival (DSS) and freedom from first-line treatment failure (FFTF). The significance of survival differences were assessed using the logrank method. Five diagnostic CHL biopsies, two tonsils and three reactive nodes SCSs were then assessed for cytokine profile. Thawed suspensions were stimulated with PMA/inomycin and stained for CD3, CD4 and either TH1-specific IL2, TNF-alpha and Ifn-g, or TH2-specific IL4, IL10, IL13 and IL21, and analysed by flow cytometry.



Results. TBET was variably expressed in the microenvironment and HRS nuclei in most patients ranging from 14-5406 cells/mm^2 (median 879). Analysis revealed that using a cutpoint of 1500 cells/mm^2 patients with higher infiltrate of TBET positive cells had superior DSS (5 year DSS 97% vs 77%; p=0.045; Figure 1) but no significant difference in OS (92%vs76%; p=0.13). Although TBET was expressed in the malignant cell in the majority of cases (>80%), with strong expression in 25%, the level of expression had no impact on outcome. TBET expression in the microenvironment was significantly higher (p=0.0033) in EBV+ cases (35%; median expression = 1448 cells/mm^2) than EBV- (median expression = 669 cells/mm^2); TH1 cytokines were expressed at high levels in CHL SCS-derived CD3+CD4+ lymphocytes in all cases: IFN-G (median=31%; range 18-66%) and TNF-A (median=26%; range 8-20%) but no detectable IL2 expression. Median TH1-cytokine expression was lower in benign samples: IFN-G=19.7% (p=ns); TNF-alpha=4.6% (p=0.03). Nuclear expression of GATA3 or CMAF was minimal in the CHL microenvironment of all cases while there was no expression of IL4, IL13 or IL21 in any CHL SCS-derived CD3+CD4+ lymphocytes despite expression in reactive nodes. **Conclusions**. We find no evidence for over-representation of TH2 in the CHL microenvironment with clear evidence for a TH1 presence, more notable in EBV-associated cases, which may have prognostic significance. This challenges the assumption of a TH2 bias being responsible for the failed immune response in CHL. Interactions between CD4+ T cells, EBV, other immune and HRS cells remains to be demonstrated functionally.

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COMPARING INTENSITY OF CHEMOTHERAPY FOLLOWED PET-GUIDED RADIOTHERAPY IN PATIENTS WITH ADVANCED STAGE HODGKIN LYMPHOMA: FINAL RESULTS OF THE GHSG HD15 TRIAL

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Background. Intensified chemotherapy with eight cycles of BEACOPP$_{escalated}$ in advanced stage Hodgkin lymphoma (HL) is highly effective but also associated with relevant treatment related toxicity. In addition, the need for radiotherapy in this setting is unclear. To reduce toxicity without losing efficacy the GHSG thus conducted the prospective randomized clinical HD15 trial investigating two less intensive chemotherapy variants, followed by PET guided radiotherapy. **Methods.** Between January 2003 and April 2008, 2182 patients with newly diagnosed, histology-proven HL aged 18-60 years were included. Patients in Ann-Arbor stage IIB with large mediastinal mass or extranodal lesions, or those in stage III or IV were randomly assigned to receive either eight cycles of BEACOPP$_{escalated}$ (8B$_{esc}$), six cycles of BEACOPP$_{escalated}$ (6B$_{esc}$), or eight cycles of BEACOPP$_{14}$ (8B$_{14}$). After completion of chemotherapy, patients in partial response (PR) with a persistent mass measuring 2.5 cm or more were assessed by PET. Only patients who were positive on centrally-reviewed PET scan received additional radiotherapy (RT) with 30Gy. The study was designed to show non-inferiority for the primary endpoint, freedom from treatment failure (FFTF).Results. The full analysis set comprised 2126 patients. Baseline characteristics were well balanced between groups. Hematological toxicities occurred in 92.4% (8B$_{esc}$), 91.7% (6B$_{esc}$), and 79.7% (8B$_{14}$) ofcases. After a median follow-up of 48 months, there were 53 deaths (7.5%) in the 8B$_{esc}$ group, 33 (4.6%) in the 6B$_{esc}$ group and 37 (5.2%) in the 8B$_{14}$ group. There were 72 secondary cancers including 29 secondary AML/MDS, 19 (2.7%) after 8B$_{esc}$, 2 (0.3%) after 6B$_{esc}$ and 8 (1.1%) after 8B$_{14}$. Complete response (CR) was achieved in 90.1% of patients after 8B$_{esc}$, in 94.2% after 6B$_{esc}$ and in 92.4% after8B$_{14}$ (p=0.01). FFTF at 5 years was 84.4% in the 8B$_{esc}$ group, 89.3% in the 6B$_{esc}$ group (97.5% confidence interval (CI) for difference 0.5% to 9.3%), and 85.4% in the 8B$_{14}$ group (97.5 CI -3.7% to 5.8%), respectively (see figure). Overall survival at five years was 91.9%, 95.3%, and 94.5%, and was also better with 6B$_{esc}$ compared to 8B$_{esc}$ (97.5% CI 0.2% to 6.5%). PFS results were similar to FFTF. PET scans performed after chemotherapy were centrally reviewed in 822 patients of whom 739 were in PR with residual mass ≥ 2.5 cm having no other exclusion criteria. 548 patients were PET-negative (74.2%) and 191 were PET-positive (25.8%). Importantly, PFS was comparable between patients in CR or those in PET-negative PR after chemotherapy with 4-year PFS rates of 92.6% and 92.1%, respectively. Only 11% of all patients in HD15 received additional RT as compared to 71% in the prior HD9 study. **Conclusions**. Six cycles of BEACOPP$_{escalated}$ followed by PET-guided RT are more effective and less toxic compared to 8 cycles in patients with advanced stage HL. In particular, critical toxicities observed with 8 cycles whereredуced with 6 cycles of BEACOPP$_{escalated}$. PET performed after chemotherapy can guide the need of additional RT in this setting and reduces the number of patients requiring RT.

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LONG-TERM FOLLOW-UP RESULTS OF AN ONGOING PIVOTAL STUDY OF BRENTUXIMAB VEDOTIN IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA (HL)

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Background. CD30 expression by Reed-Sternberg cells is a defining feature of HL. Brentuximab vedotin comprises an anti-CD30 antibody conjugated by a protease-cleavable linker to MMAE, a microtubule-disrupting agent. Brentuximab vedotin selectively induces apoptotic death of CD30+ cells by binding, internalizing, and releasing MMAE. **Aims.** A pivotal phase 2 study was conducted to determine the efficacy and safety of brentuximab vedotin in patients (pts) with relapsed or refractory HL after autologous stem cell transplant (auto-SCT) (ClinicalTrials.gov #NCT00848926); long-term follow-up data from this ongoing trial are presented.

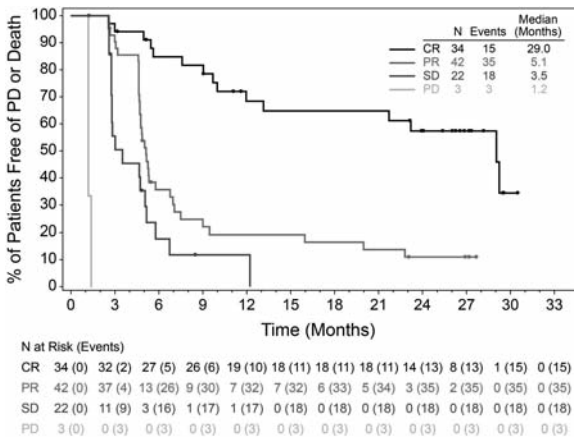

Figure 1. Progression-free Survival by Best Clinical Response.

Methods. The primary endpoint was the objective response rate (ORR) per independent review according to the Revised Response Criteria for Malignant Lymphoma (Cheson 2007). Pts received 1.8 mg/kg brentuximab vedotin every 3 weeks as a 30-minute outpatient IV infusion for up to 16 cycles. Informed consent was obtained for all patients. **Results.** 102 pts were enrolled; 53% were female and the median age was 31 yrs (range, 15-77 yrs). Pts had received a median of 3.5 (range 1-13) prior cancer-related systemic therapies excluding auto-SCT. 71% of pts had primary refractory disease and 42% had not responded to their most recent prior therapy. As previously reported, the ORR was 75% (76 of 102 pts) with complete remissions (CRs) in 33% of pts (n=34). At the time of this analysis (January 2012), the median observation time from first dose was 26.5 months (mths; range, 1.8 to 30.9 mths). 38 of 102 pts have died and the median overall survival has not yet been reached. The estimated 24-mth survival rate was 65% (95% CI: 55%, 74%). The median progression-free survival (PFS) for all pts was 5.6 mths (range, 1.2 to 30.5+ mths). 34 pts obtained a CR with brentuximab vedotin; 13 remain in CR and have not started a new anti-cancer therapy since discontinuing study treatment other than 3 who received prophylactic allo-SCT following brentuximab vedotin while in CR. The PFS for these 13 pts ranged from 23.1+ to 30.5+ mths. The median PFS for pts who obtained a CR was 29.0 mths, which was notably longer than the median PFS for patients who obtained a partial remission (PR; 5.1 mths) or stable disease (SD; 3.5 mths). As previously reported, the most common (≥15%) treatment-related adverse events (AEs) of any grade were peripheral sensory neuropathy, nausea, fatigue, neutropenia, and diarrhea. AEs ≥ Grade 3 occurring in ≥5% of pts were neutropenia, peripheral sensory neuropathy, thrombocytopenia, and anemia. **Conclusions.** 34 of 102 heavily pretreated pts (33%) with relapsed or refractory HL obtained a durable CR with brentuximab vedotin treatment. More than a third of the pts who obtained a CR remain in CR at the time of this long-term follow-up analysis. The median PFS for pts who obtained a CR was 29.0 mths, which was notably longer than the median PFS for patients who obtained a PR or SD (5.1 mths and 3.5 mths, respectively).

1110

OUTCOME OF PATIENTS WITH EARLY STAGE HODGKIN LYMPHOMA ACCORDING TO GHSG AND NCIC-CTG RISK CLASSIFICATION: THE PRINCESS MARGARET HOSPITAL EXPERIENCE

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Background. Early stage Hodgkin lymphoma (HL) carries a very favourable prognosis; nevertheless, a subset of patients progress after first-line therapy, and others achieve cure at the expense of considerable treatment-related toxicity. Risk-adapted therapy has been tested, aimed at minimizing toxicity for those with favourable HL. Criteria used to identify high risk patients were developed at a time when sub-total nodal irradiation was standard for early stage HL, questioning their relevance in the modern era of combined modality therapy (CMT). **Aims.** We wished to determine whether the German Hodgkin Study Group (GHSG) or National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) stratification criteria for early stage HL could discriminate between low and high-risk patients treated with doxorubicin-based CMT, in terms of overall (OS) and progression-free survival (PFS). **Methods.** We performed a retrospective review of 495 stage I-II HL patients diagnosed and treated at PMH from 1997 to 2009. Patients were stratified into early favourable, early unfavourable or advanced groups using GHSG and NCIC-CTG risk factors, and their OS and PFS assessed by group.

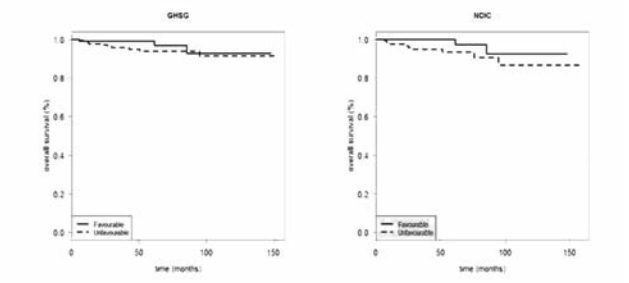

Figure 1. Kaplan-Meier estimates of overall survival probabilities by GHSG and NCIC-CTG classification schemes.

Results. Before multiple imputation to complete missing data, 21%, 44.6% and 10.3% of patients were classified as early favourable, early unfavourable and advanced, respectively, according to GHSG criteria (24% unclassifiable due to missing data); similarly 18.6%, 32.3%, 38.6% and 10.5% had early favourable, early unfavourable, advanced or unclassifiable HL, respectively, by NCIC-CTG criteria. After a median follow up of 37.4 months, OS was 94.4% and PFS 88.1%. After controlling for treatment intensity, there was no statistically significant difference in OS or PFS between early favourable and early unfavourable groups as defined by either criteria set. Hazard ratios (HR) for death and progression were 1.779 (95% CI -0.422, 1.573; p=0.256) and 1.327 (-0.396, 0.961; p=0.410), respectively, for unfavourable compared with favourable patients by GHSG stratification, while HRs by NCIC-CTG criteria for death and progression were 3.556 (-0.286, 2.823; p = 0.110) and 1.451 (-0.560, 1.304; p = 0.426). Estimated 10-year OS for early favourable and early unfavourable patients by GHSG criteria was 92.8% (95% CI 0.762, 0.979) and 91.3% (0.827, 0.958), respectively, while PFS was 87.8% (0.739, 0.945) and 77.7% (0.637, 0.868). Similar estimates were seen for patients categorized by NCIC-CTG criteria: OS for favourable patients was 92.4% (0.714, 0.981) vs 86.5% (0.719, 0.938) for unfavourable, and PFS was 85.9% (0.693, 0.939) and 71.8% (0.499, 0.854), respectively. Patients with advanced disease by both criteria had significantly inferior OS relative to favourable patients, while PFS, although inferior, was only marginally significant. Exploration of individual prognostic factors defined by GHSG and NCIC-CTG criteria revealed extranodal disease and age > 40 predicted OS, while extranodal disease, elevated ESR and bulky mediastinal disease were predictive of PFS. **Conclusions**. Although individual risk factors carry prognostic relevance, previously proposed

criteria may not identify patients at increased risk of treatment failure or death. As these criteria are increasingly used to deliver risk-adapted therapy, further studies are needed to identify additional, biologically meaningful prognostic factors for early stage HL.

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TREATMENT-RELATED MORTALITY IN PATIENTS UNDERGOING THERAPY FOR ADVANCED HODGKIN'S LYMPHOMA: ANALYSIS OF THE GERMAN HODGKIN STUDY GROUP (GHSG)

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Background. The outcome of patients with Hodgkin's Lymphoma (HL) has improved over the past decades. Even patients with advanced-stage HL show a 10 year freedom from treatment failure (FFTF) of 82% and an overall survival (OS) of 86% when treated with eight cycles of $BEACOPP_{escalated}$ (Bleomycin, Etoposid, Adriamycin, Cyclophosphamide, Vincristine, Procarbacine, Prednisone). However, $BEACOPP_{escalated}$ has been associated with some acute and long-term toxicity, including a concern for treatment related mortality (TRM). **Aims.** The objectives of this study were to identify possible risk factors for TRM and help improve supportive-care strategies in advanced stage HL patients undergoing $BEACOPP_{escalated}$, thus reducing TRM and improving overall survival in these patients. **Methods.** We investigated the incidence of TRM and possible risk factors in a retrospective analysis of 3402 patients with advanced HL who were treated with $BEACOPP_{escalated}$ within the GHSG HD9, HD12 and HD15 multicenter clinical trials between 1993 and 2008. **Results.** The overall TRM rate was 1· 9% (HD9 = 6/439, HD12 = 38/1569, HD15 = 20/1394). TRM occurred during the first course of $BEACOPP_{escalated}$ in 20 cases (31· 3%). The median age of patients who died from TRM was 50 years (17 to 64), as compared to 33 years (16 to 65) in the whole group of analyzed patients. Most common causes of TRM were neutropenic infections, either bacterial or (n=56, 87· 5%). Univariate analyses of possible risk factors revealed that TRM was six times increased in patients older than 40 years, four times increased in patients with a poor performance status (ECOG=2 or Karnofsky<80) and three times increased in patients with an International Prognostic Score (IPS) of ≥3 compared with patients without these risk factors, respectively. Gender, stage of disease, B-symptoms, and well-known risk factors such as 3 or more involved lymph node areas, extranodal involvement, large mediastinal tumor or elevated ESR were not associated with an increased risk of TRM. In a multivariate analysis of TRM and all mentioned risk factors, age and poor performance status were the only significant prognostic factors of TRM. A particularly higher risk of TRM is seen in patients between 40 and 50 years of age with poor performance status as well as in patients who are 50 years and older. According to the identified risk factors age and performance status, it is possible to define a risk score for TRM ranging from zero to three. Patients with a TRM-Score of ≥2 have an increased risk for TRM. **Conclusions.** Since important risk factors for TRM could be identified in our study, possible measures should be taken to reduce toxicity with $BEACOPP_{escalated}$ in patients with these risk factors. Such measures include a pre-phase treatment of patients over 40 years of age, inpatient treatment of patients with risk factors at least for the first course of $BEACOPP_{escalated}$, an earlier administration of G-CSF starting from day 4 of every cycle instead of day 8 as was previously the case, as well as the prophylactic use of antibiotics during the whole chemotherapy period. These measures have currently been implemented in the GHSG ongoing trial HD18 for advanced stage HL.

Non-Hodgkin's lymphoma - Clinical

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INCIDENCE, CHARACTERISTICS AND OUTCOME OF CNS INVOLVEMENT IN MANTLE CELL LYMPHOMA: A MULTICENTRE RETROSPECTIVE ANALYSIS FROM THE EUROPEAN MANTLE CELL LYMPHOMA NETWORK

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Background. CNS involvement in Mantle Cell Lymphoma (MCL) is an uncommon but devastating event. Limited data exist to define true incidence or clinical features. **Aims**. To describe the clinical characteristics and outcomes of patients with CNS involvement in Mantle Cell Lymphoma. **Methods.** The study design was a multicentre retrospective case series. 12 participating institutions provided 1) total number of patients in their MCL database, 2) number with CNS involvement and 3) detailed information including demographics, baseline characteristics, presentation, prior treatments, investigations, subsequent therapies and outcomes. **Results.** 1314 patients with MCL were included in this analysis. 53 patients with CNS involvement were identified between 1988 and 2011. The crude cumulative incidence of CNS involvement was 4.0%. Of those with CNS disease, 71% were male, median age 61 years (38 - 82). At diagnosis, 36 (68%) had classical MCL, 15(28%) blastoid histology. Among 26 patients with data, Ki67 value by immunohistochemistry was 60% or higher in 12 cases (46%). 91% of patients were stage IV, 52% had B symptoms at presentation, and the median white cell and lymphocyte counts were 17.5 x 10^9/L and 12.2 x10^9/L respectively. Among 24 patients with data, high MIPI score (6 or more) was seen in a striking 18(75%). 9 patients had CNS involvement at diagnosis. The commonest neurological symptoms were weakness (26%), altered mental state (25%), ocular/diplopia (21%) and headache (19%). Sensory disturbances were less common. 5% were asymptomatic. Lumbar puncture was performed in 46 patients, with 85% positive by cytology. Flow cytometry improved the yield to 91% in the 33 patients tested. Radiographic changes suggestive of CNS disease were seen in 36/44 (82%) cases performed, with leptomeningeal changes was present in 15/36 (42%) and cortical lesions in 11/36 (31%).Prior therapies were heterogeneous with a median of 2 prior treatments (0 - 6), the commonest regimen being (R)-CHOP like in 26 (49%) with 16 (30%) receiving CNS penetrating doses of methotrexate and cytarabine ie (R)-Hyper CVAD, R-maxiCHOP/high dose cytarabine). 18/45 (40%) patients with data received prior intrathecal prophylaxis. 36 (68%) of patients had concurrent systemic relapse at the time of their CNS relapse, with 15 (28%) having isolated CNS relapse. The median time to CNS relapse from diagnosis was 11.5 months, with 67% occurring within 2 years of diagnosis. 38 (72%) received chemotherapy alone as treatment for their CNS relapse, with 5 (9%) receiving combined modality incoroporating chemotherapy with radiotherapy. A further 5% were not offered active treatment. There was a predictably poor outlook once diagnosed (median OS 3.3 months) although 9 patients remain alive at the time of reporting (median survival from CNS diagnosis 3.9 months, range 0.9 to 63.2 months). **Conclusions**. This is the largest dataset describing CNS involvement in Mantle Cell Lymphoma with an estimated cumulative incidence of 4%. In particular, high MIPI score and high Ki67 were notable features. Most CNS relapse occurred within the first two years., suggesting that if high risk features can be targeted CNS prophylaxis as part of initial therapy may be effective in preventing CNS progression.

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RISK FACTORS FOR CNS RELAPSE/ PROGRESSION IN PATIENTS WITH AGGRESSIVE B-CELL LYMPHOMA: THE ROLE OF EXTRANODAL SITES

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Background. We recently reported on frequencies and risk factors for CNS relapse or progression in elderly (Boehme et al., BLOOD 2009: 113, 3896f) and younger patients (Schmitz et al., Ann. Oncol. 2011: doi:10.1093) with aggressive B-cell lymphoma. **Aims.** With this study we wanted to address the question if involvement of distinct extranodal sites increased the risk for CNS relapse or progression. **Methods.** Four thousand four hundred seventy patients aged 18-80 years with newly diagnosed aggressive B-cell lymphoma were analyzed. All patients were treated on trials of the German High-Grade Lymphoma Study Group (DSHNHL) or the Mabthera International trial (MInT). Systemic first-line therapy consisted of 4 - 8 courses of CHOP+/-etoposide+/-rituximab. Cycle number and doses of cytotoxic agents varied according to patients` age and the individual risk factor profile defined by the International Prognostic Index (IPI).Results. 2494 patients (55.8%) were male, median age was 60 years, 2404 patients (53.8%) presented with stage I-II disease, IPI was 0, 1 in 2295 patients (51.4%) and 864 patients (19.3%) showed more than 1 E-lesion. 154 patients (3.4 %) developed meningeal and/ or parenchymal CNS relapse or progressed in the CNS during therapy. Uni- and multivariate analyses were performed in order to address the question if involvement of distinct organs or sites indicated an increased risk of CNS disease. In univariate analyses involvement of lungs, liver, bone, pleura, gastrointestinal tract, paranasal sinuses, breast, kidney, adrenals, peritoneum, bladder, ovary, soft tissue and skin was associated with an increased risk of CNS disease. Using multivariate analysis adjusted for the IPI factors (LDH>N, ECOG>1, advanced stage, age>60years, number of extranodal sites involved) involvement of the following organs/ sites remained significant: skin (relative risk 3.7, p<0.001), paranasal sinuses (RR 3.0, p=0.001), breast (RR 2.9, p=0.011), kidney (RR 2.4, p=0.003), adrenals (RR 2.4, p=0.006), and testes (RR 2.2, p=0.039) whereas other organs/ sites (e.g. bone marrow, orbita, salivary glands) involvement of which is often considered as a risk factor for CNS relapse did not significantly increase the risk of CNS disease. **Conclusions**. Patients with elevated LDH, B-symptoms, and involvement of more than 1 extranodal site have been reported to carry an increased risk for CNS relapse or progression. Analysing > 4400 study patients we show that involvement of certain extranodal sites (paranasal sinuses, breast, kidney, adrenals and testes) significantly increased this risk while other sites traditionally considered to increase CNS disease were not found significant. These findings will have important clinical implications when considering future strategies which patients with aggressive B-cell lymphoma should be candidates for CNS prophylaxis.

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ROLE OF RADIOTHERAPY FOR ELDERLY DLBCL PATIENTS IN THE RITUXIMAB (R) ERA: FINAL RESULTS OF THE RICOVER-60-NO-RX STUDY OF THE DSHNHL

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Background. 117/306 (38%) of the total of 1222 elderly (61-80 y) DLBCL pts. treated in RICOVER-60 with 6xR-CHOP-14+2R were assigned to receive additional radiotherapy (Rx) to bulky disease (*Pfreundschuh et al., Lancet Oncol. 2008*). **Aims.** To study the relevance of Rx to bulky disease (Bx) in elderly DLBCL patients. **Methods.** 166 pts. were prospectively treated without Rx in the R-CHOP-14-noRx amendment of RICOVER-60. the outcome of these 166 R-CHOP-14-noRx patients was compared with the 306 patients who had received 6xR-CHOP-14+2R plus radiotherapy to Bx (³7.5 cm) in RICOVER-60. **Results.** 164/166 R-CHOP-noRx patients are evaluable (median observation: 39 mos). Patients in R-CHOP-noRx were older (71 vs. 69 y.; median; p=0.018), more frequently in advanced stages (60% vs. 50%; p=0.037), and with extranodal involvement (63% vs. 53%; p=0.024), while Bx was more frequent in R-CHOP-14-Rx (38% vs. 29%; p=0.038). Overall response to therapy, EFS and OS were similar in the two studies adjusting for the prognostic imbalances between the cohorts. Patients with Bx who received received additional radiotherapy to Bx in R-CHOP-14-Rx had a better 3-year EFS (80% vs. 54%; p=0.001), a better PFS (88% vs. 62%; p<0.001), and a better OS (90% vs. 65%; p=0.001) compared to R-CHOP-14-noRx. This was due the worse outcome of pts. with Bx in R-CHOP-14-noRx not achieving CR or CRu after 6xR-CHOP, since there was no difference in 3-year EFS in patients with Bx in CR or CRu after 6xR-CHOP-14 with and without additional radiotherapy (3-year EFS and PFS: 84% vs. 75%; p=0.430); OS 87% vs. 79%; p=0.839). **Conclusions.** In the R era, radiotherapy to bulky disease does not improve the outcome of elderly pts. in CR/CRu after completion of R-CHOP-14 immunochemotherapy, but appears to be beneficial for pts. with Bx not achieving CR/Cru. By restricting Bx radiotherapy to patients not achieving a CR/CRu, 43% of the patients with Bx could be spared radiotherapy. *Supported by Deutsche Krebshilfe.*

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R-CHOP21 VS R-CHOP14 IN 712 DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: RESULTS OF A MULTICENTRIC RETROSPECTIVE STUDY FROM ITALIAN LYMPHOMA FOUNDATION (FIL)

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Background. Diffuse large B cell lymphoma (DLBCL) is the most common histotype of non-Hodgkin's lymphoma. R-CHOP21 (C21) is considered the standard therapy but a large number of studies tested the dose dense regimen R-CHOP14 (C14). **Aims.** The aim of our multicenter retrospective study was to evaluate the efficacy in terms of Overall Survival (OS) and Progression free survival (PFS) of the two regimens C21 and C14 in a large cohort of patients (pts) with a diagnosis of DLBCL or follicular grade IIIb lymphoma, treated with curative intent. **Methods.** We evaluated 712 pts treated in seven Italian Haematology Departments from January 2002 to December 2010, 478 treated with C21 and 234 treated with C14. The two cohorts of pts were balanced for all clinical characteristics excepted age higher than 65 years (54% in C21 vs 26% in C14 (p 0.000)), involvement of more than three nodal stations (37% C14 vs 27% C21 (p 0.005)), and bone marrow involvement (21% in C14 vs 15% C21 (p 0.05)). All pts in C14 used primary prophylaxis with G-CSF.

Overall Survival	CHOP21 p value	CHOP14 p value
Age > 64 years	.0000	.01
Stage III-IV	.001	.0001
Systemic symptoms	.0000	n.s.
Abnormal LDH value	.0000	.0002
Marrow positivity	.01	n.s.
> 3 stations involved	.0000	n.s.
> 1 extranodal site	.0000	n.s.
High IPI	.0000	.001
Progression Free Survival	**CHOP21 p value**	**CHOP14 p value**
Age > 64 years	.001	n.s.
Stage III-IV	.0000	.0000
Systemic symptoms	.0000	n.s.
Bulky disease	.009	n.s.
Abnormal LDH value	.0000	.0000
Marrow positivity	.0000	n.s.
> 3 stations involved	.0000	n.s.
> 1 extranodal site	.0000	n.s.
High IPI	.0000	.001

Results. After induction therapy 554 pts (78%) obtained a complete remission: 367/478 (77%) after C21 and 187/234 (80%) after C14. After a median period of observation of 36 months 72 pts out of 554 CR pts relapsed, 46 (46/367: 12,5%) in the C21 arm and 26 (26/187: 14%) in the C14 arm. OS was 80% in C21 and 83% in C14 (p:n.s.); PFS was 77% in C21 and 76% in C14 (p:n.s.). Univariate statistical analysis showed that OS was significantly superior in younger pts (<65 year), Ann Arbor stage I-II, absence of B-symptoms, no bulky disease, normal LDH value, negative bone marrow biopsy, low and low-intermediate risk IPI, less than four nodal stations involved and less than two extranodal site; PFS was significantly superior for the same characterstics except bulky disease. Multivariate analysis showed that OS was affected by age (p .002) and IPI (p .0000) and PFS by stage (p .002) and IPI (p .0000). The results of univariate analysis performed

stratifying for therapy are shown in table1.As expected, haematological grade III/IV toxicity was more frequently observed in pts treated with C14. No differences in extra-haematological toxicities were observed; three deaths for sepsis were observed (1 in C14 and 2 in C21). **Conclusions**. Our results confirm that C14 does not improve either OS or PFS in comparison with standard C21 in the whole lymphoma population analysed. However, we observed in univariate analysis that the intensified therapy reduced the prognostic impact on OS and PFS of important factors such as bone marrow involvement and high tumor burden in comparison with standard C21, suggesting that C14 should be useful in higher risk pts (e.g. marrow positivity or high tumor burden at diagnosis). Due to the retrospective nature of this study these results should be confirmed in prospective multicentric studies.

1116

CHLAMYDOPHILA PSITTACI ERADICATION WITH DOXYCYCLINE IS AN ACTIVE FIRST-LINE TARGETED THERAPY FOR OCULAR ADNEXAE LYMPHOMA: FINAL RESULTS OF AN INTERNATIONAL PHASE II PROSPECTIVE STUDY ((IELSG#27 TRIAL)

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Background. The pathogenic association between *Chlamydophila psittaci (Cp*) infection and ocular adnexal marginal zone lymphoma (OAMZL) has been demonstrated in some geographical areas; with several complementary evidences; the efficacy of chlamydial eradication with doxycycline monotherapy has been associated with lymphoma regression in half of these patients. However, clinical and therapeutic knowledge on this association mainly result from retrospective series with variations in stage, management and follow-up duration. **Aims.** This is the first phase II trial aimed to elucidate *Cp* prevalence and activity of first-line doxycycline in lymphoma regression and *Cp* eradication in patients with stage-I OAMZL (ClinicalTrial.gov NCT01010295). **Methods.** Forty-seven patients with stage-IEA OAMZL were registered. Tumor tissue, conjunctival swabs and peripheral blood from 44 patients were assessed for seven Chlamydiaceae infections by three PCR protocols. Thirty-four patients with measurable/parametrable disease were treated with doxycycline 100 mg bid for 21 days and assessed for chlamydial eradication and lymphoma response (primary endpoint). Bacterial eradication was tested at basal time, at 3 and 12 months after antibiotics on swabs and PBMC. Response assessment was assessed at the same timepoints, and every six months afterward. **Results.** *Cp* DNA was detected in biopsies of 39 (89%) patients; no other chlamydiaceae were detected. *Cp* DNA was detected in 97% (n= 38) of baseline conjunctival swabs and in 69% (n= 27) of PBMC samples of the 39 *Cp*-positive OAMZL; no cases with *Cp*-negative biopsy disclosed *Cp* DNA in their swabs or PBMCs. Twenty-two (92%) of the 24 patients who reported history of prolonged contact with household animals had a *Cp*-positive lesion.Twenty-nine patients had *Cp* DNA in baseline swabs and/or blood samples and were evaluable for chlamydial eradication, which was achieved in 14 (48%) patients. Lymphoma regression after doxycycline treatment was complete in six patients and partial in 16 (ORR=65%, 95%CI:49-81%); 11 had SD and one PD. Nineteen of the 29 patients with *Cp*-positive OAMZL achieved an objective response (ORR: 66%; 95%CI: 50-82%). Lymphoma regression was achieved in 12 of the 14 *Cp*-eradicated patients and in seven of the 15 non-eradicated patients (86% vs. 47%; p=0.02).At a median follow-up of 37 months (range 15-62), 20 patients remain relapse-free (5-year PFS: 55±9%). Fifteen of the 22 patients with responsive lymphoma were relapse-free at the time of analysis, with a median TTP of 30+ months (range 8 - 62+). None of the patients who achieved CR experienced relapse after 36-60 months. Tumor failure occurred in four of the 14 *Cp*-eradicated patients and in eight of the 15 non-eradicated patients, with a 5-yr PFS of 68±13% and 47±13% (p=0.11), respectively. No patient died of lymphoma, two deaths due to stroke and unrelated sudden death were recorded. **Conclusions.** Upfront doxycycline is a rational and active treatment for patients with stage-I, *Cp*-positive OAMZL. Lymphoma regression is consequent to *Cp* eradication, which can easily be monitored on conjunctival and blood samples. The definition of mechanisms of resistance to doxycycline and the design of more effective administration schedules are warranted.

Autologous stem cell transplantation

1117

STRONG PROGNOSTIC VALUE OF ETOPOSIDE PHARMACOKINETICS IN LYMPHOMA PATIENTS TREATED WITH BEAM REGIMEN AND ASCT: MULTICENTER STUDY OF GROUPE D'ETUDES DES LYMPHOMES DE L'ADULTE (GELA)

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Background. Relationships between pharmacokinetic (PK) parameters of etoposide and toxicity-survivals were reported in solid tumor patients treated at standard doses [1]. When given at high dose in lymphoma patients, etoposide PK may have a strong influence on outcomes. **Aims.** The prospective LYMPK study primary objective was to assess the impact of etoposide pharmacokinetic (PK) parameters on toxicity & efficacy in lymphoma patients receiving the BEAM regimen (carmustine, cytarabine, etoposide and melphalan) followed by autologous stem cell transplant (ASCT). We previously showed the high inter-individual variability in etoposide PKs, defined by area under the curve (AUC) and trough concentration (Cmin), among study patients treated with the same doses /m2 [2]. **Methods.** Ninety-six patients with malignant lymphoma [Non-Hodgkin lymphoma (NHL): 84; Hodgkin lymphoma (HL): 12] at 1st line (n=52) or relapse (n=44) were enrolled in 5 centers. All received BEAM regimen, including high dose etoposide (100 to 200 mg/m2 bid for 4 days), followed by ASCT. Individual etoposide AUC and Cmin were estimated by population PK approach using NONMEM® program. The impact of PK parameters on toxicity and survival was assessed using linear regression and univariate/multivariate analyses.

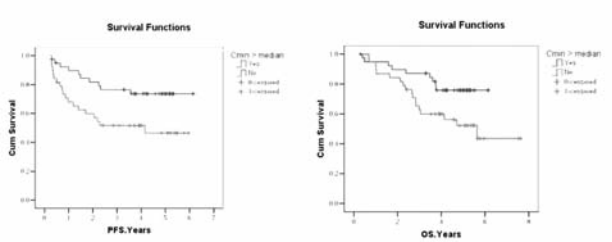

Figure 1. Impact of etoposide residual concetration (Cmin) on PFS and OS.

Results. Data from 90 patients (NHLs: 78; HLs: 12) were assessable after a 4.2 year median follow-up. The bi-compartment model previously reported was used to characterize PK parameters [2]. Etoposide AUC and Cmin correlated with mucositis duration, especially for grade 3-4 toxicity ($p< 0.05$), but not with other toxicities. In NHL patients, Cmin categorized by the median had significant prognostic value regarding 5 year progression free survival (PFS) (73.6% vs 46.5%, if Cmin > median, p=0.01) and 5 year overall survival (OS) (76% vs 52%, if Cmin > median, p=0.03). When assessed with available IPI prognostic factors (age; performance status; LDH & stage) using Cox analysis, Cmin was the only significant independent prognostic factor regarding PFS (HR = 0.39, 95% CI = 0.18-0.86); disease specific survival (HR = 0.35, 95% CI = 0.14 - 0.91) and OS (HR = 0.43, 95% CI = 0.19 - 0.96) in NHL patients. Etoposide AUC was also a significant independent factor against IPI prognostic factors using Cox analysis factor adjusted on lymphoma type (HL vs NHL) regarding disease specific survival (HR= 0.38, 95% CI = 0.16-0.93) and overall survival (HR = 0.46, 95% CI = 0.21-0.98). **Conclusions.** LYMPK study results suggest that individual etoposide pharmacokinetic parameters, especially systemic exposure and residual concentration, have strong impacts on survival in lymphoma patient receiving BEAM regimen and ASCT. Given the high variability in patient pharmacokinetics [2], plasma concentration-based adjustment of etoposide dose may be considered in future studies.

[1] You B. *et al*, *Lung Cancer 2008*;62:261-72.
[2] You B. *et al*, Proc. *ASCO 2008*

1118

RANDOMIZED STUDY OF IBRITUMOMAB TIUXETAN (ZEVALIN) & HIGH-DOSE BEAM CHEMOTHERAPY (Z-BEAM) VS BEAM ALONE PRIOR TO AUTOLOGOUS TRANSPLANTAT IN AGGRESSIVE LYMPHOMA; LONG-TERM FOLLOW-UP AND SUBSET ANALYSIS

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Background. High-dose chemotherapy and autologous stem-cell transplantation (ASCT) is the standard therapy for patients with refractory/relapsed aggressive lymphoma. In the era of rituximab-containing front-line regimens, it is becoming more challenging to salvage patients in this setting and novel approaches are required. **Aims and Methods.** We have conducted a multi-center, prospective randomized study comparing the outcomes following ASCT with high-dose BEAM chemotherapy compared to BEAM combined with standard-dose Zevalin (0.4 mCi/kg) administered on day-14 prior to ASCT in patients with aggressive lymphoma. The primary end-point was 2-year overall survival (OS). Initial results showed the feasibility and safety of this approach and suggested a possible survival advantage. The current analysis is a long-term follow-up with all surviving patients reaching the primary end-point.*Results.* The study included 43 patients, median age 55 years (range, 23-67). Thirty-four patients had diffuse large B-cell lymphoma (DLBCL), refractory to first-line therapy (n=6) or recurrent (n=28). Nine patients had transformed follicular lymphoma. All patients responded to second-line therapy, however 16 had positive PET-CT prior to ASCT. Twenty-six patients were scored as high-risk disease if they had a short initial remission (<12 months) or high IPI score (3) at relapse, while 17 patients were scored as low-risk disease. In all, 22 patients were randomized to Z-BEAM and 21 to BEAM alone. Patient and disease characteristics were well matched between the groups and there was no difference in engraftment kinetics or toxicity profile. The median follow-up is 39 months (range, 24-60). The 2-year OS is 91% (95%CI, 79-100%) and 61% (95%CI, 39-82%) after Z-BEAM and BEAM, respectively (p=0.03). Similar results were observed when the analysis was limited to the 34 patients with DLBCL (94% Vs 54%, p=0.01). The 2-year progression-free survival (PFS) was 59% (95%CI, 39-80%) and 42% (95%CI, 20-63%), respectively (p=NS). Advanced age (55 years) was associated with worse outcome; 2-year OS been 90% and 64% in the younger and older subgroups, respectively (p=0.07). Z-BEAM was beneficial in both subgroups, but more so in the older subgroup. 2-year OS was 85% and 33% in the older group (p=0.02) compared to 100% and 83% in the younger subgroup (p=0.20), respectively. Similarly, Z-BEAM was beneficial in high-risk disease, 86% Vs 58% (p=0.11) and low-risk disease, 100% Vs 67% (p=0.08), and in patients with positive PET-CT prior to ASCT, 82% Vs. 50% (p=0.20) or negative PET-CT, 100% Vs. 71% (p=0.06). Multivariable analysis identified advanced age [HR 7.2 (p=0.02)], high-risk disease [HR 4.2 (p=0.08)] and conditioning with BEAM alone [HR 10.1 (p=0.006)] as poor prognostic factors for 2-year OS. The same factors were also prognostic for 2-year PFS with HR of 10.2 (p=0.002), 2.5 (p=0.07) and 2.7 (p=0.04), respectively. Positive PET-CT had borderline statistical significance, HR 2.3 (p=0.09).*Conclusions*: Standard-dose Zevalin combined with BEAM high-dose chemotherapy is safe and possibly more effective than BEAM alone as conditioning regimen for ASCT in the era of rituximab-containing chemotherapy regimens. Based on these data, a consortium of centers was formed to validate these conclusions in a larger international phase-3 randomized trial.

1119

THIOTEPA-BASED HIGH-DOSE PREPARATION VS BEAM FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AUTOHSCT) IN LYMPHOMA OTHER THAN PCNSL: A RETROSPECTIVE STUDY FROM THE EBMT

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Background. Thiotepa is an alkylating agent approved for high-dose therapy for autologous HSCT. Because of its excellent capacity to cross the blood-brain barrier, it is regularly used for autoHSCT for primary CNS lymphoma (PCNSL). However, although thiotepa-based myeloablation might have benefits over traditional BEAM preparation because of better brain availability and less pulmonary toxicity, clinical level information about thiotepa-based autoHSCT outside of the PCNSL field is sparse. The purpose of the present retrospective study was to provide information on the potential risks and benefits of thiotepa-based preparative regimens in autoHSCT for distinct subtypes of lymphoma outside of the PCNSL setting. **PRIMARY OBJECTIVE** was to compare the outcome of thiotepa-based autoHSCT (TT) with that of BEAM autoHSCT (BEAM) separately for diffuse large B-cell lymphoma (DLBCL; excluding PCNSL), follicular lymphoma (FL), peripheral T Cell lymphoma (PTCL) and Hodgkin's lymphoma (HL). **PRIMARY ENDPOINT** was progression-free survival (PFS), secondary endpoints were overall survival (OS), non-relapse mortality (NRM), and incidence of relapse (IR). **ELIGIBLE** were patients >18 years from 12 European countries who were registered with the EBMT and had received TT-based myeloablation or BEAM for a first autoHSCT between 2003-2010 for FL, DLBCL, PTCL, or HL, and had Med A level information available. **STATISTICAL ANALYSIS** was based on a 1:2 matched pair comparison using stratified Cox and Fine & Gray regression models for comparison. Matching factors were age (+/- 10 years), sex, lymphoma subtype, time from diagnosis to autoHSCT, remission status at autoHSCT, and year of autoHSCT. **results.** 420 patients with TT fulfilled the inclusion criteria and were matched with 816 BEAM patients (24 patients had only one match). Of these 1236 patients, 67% had DLBCL, 8% FL, 3% PTCL, and 22% HL. Remission status at autoHSCT was CR/PR1 in 42%, CR/PR >1 in 28%, more advanced disease in 19%, and unknown in 11%. The TT group contained a significantly larger proportion of patients with poor performance status (12% vs 6%, p 0.0011). Because of the low numbers PTCL were excluded from endpoint analyses. The hazard ratio for PFS with TT vs BEAM inDLBCL,FL, and HL was 1.31 (0.91-1.89), 0.65 (0.19-2.2), and 0.86 (0.49-1.51). Similarly, no significant differences between TT and BEAM became evident in any lymphoma subset for OS, NRM, and IR. **Conclusions.** Despite the unfavourable imbalances in performance status, we found no evidence for inferiority of TT-based compared to BEAM autoHSCT forDLBCL,FL, and HL. Thus, in these lymphoma subtypes TT-based high-dose therapy might be a valuable alternative to BEAM in situations were BEAM is unavailable or contraindicated.*Supported by a restricted grant from Riemser AG, Greifswald, Germany.*

1120

PLERIXAFOR FOR FIRST LINE STEM CELL MOBILIZATION PRODUCES GREATER STEM CELL YIELDS THAN CONVENTIONAL CHEMOTHERAPY: AN INTERIM ANALYSIS OF THE PHANTASTIC TRIAL

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Background. Plerixafor is an effective and well tolerated agent for mobilising blood stem cells in patients who fail mobilisation with conventional chemotherapy schedules. It has a product license for second line use in myeloma and lymphoma patients. However, its place as a first line stem cell mobilisation agent is unclear. The PHANTASTIC study (ClinicalTrials.gov Identifier: NCT01186224) is examining first line plerixafor with G-CSF in 100 patients with underlying myeloma or lymphoma requiring autologous transplantation. **Aims.** In a planned interim analysis of the first 60 patients, to determine whether $CD34^+$ yields after first line plerixafor are superior to those attained with conventional chemotherapy + G-CSF in historical controls. **Methods.** All entrants received G-CSF at ~5ug/kg on days 1-4, and plerixafor on day 4, at a dose of 240 ug/kg if creatinine clearance >50 mls/min (59 cases) or 160 ug/kg if creatinine clearance 30-50 mls/min (1 case). Stem cell harvesting was performed on days 5-8 with additional daily G-CSF and plerixafor as needed, until the target yield of 4×10^6 $CD34^+$ cells /kg recipient weight was collected. Results were compared with 270 consecutive historical control patients mobilised by conventional chemotherapy (mostly cyclophosphamide 1.5gm/m2) + G-CSF. All entrants and controls were treated at our institution.**Results.** 26 patients had underlying myeloma, 26 Non-Hodgkin lymphoma and 8 Hodgkin's disease. No serious adverse events were seen during or in the 21 days following harvesting completion,. Ten patients developed mild transient gastrointestinal symptoms, insomnia and headaches which were possibly plerixafor-related.48 (80%) of the 60 plerixafor mobilised patients achieved the primary composite endpoint of BOTH an adequate stem cell harvest ($^3 4 \times 10^6$ $CD34^+$ cells/kg in ≤ 2 aphereses) AND no evidence of a neutrophil count < 1×10^9/ L over the 3 weeks after starting mobilisation. This is significantly greater than the 100 (37%) of 270 historical control patients who achieved this endpoint ($p < 0.0001$). Plerixafor also achieved a significantly greater median $CD34^+$ cell yield than in 235 assessable historical controls (median 5.49 vs. 3.89×10^6 /kg; $p < 0.001$). All plerixafor treated patients met the target $CD34^+$ cell dose within 4 harvests, whereas 135 of 270 assessable historical controls failed to meet this target, 18 of whom required either marrow harvesting or an additional round of stem cell mobilisation.To date, 49 of the 60 plerixafor mobilised patients have undergone transplantation and all these are assessable for engraftment. The median time to neutrophil engraftment of 0.5×10^9/L was 12 days, and for platelet engraftment to 50×10^9/L was 21 days. 23 cases are assessable at 12 months post transplant, of whom 1 has died and 3 have relapsed; the remaining 19 are alive and well. **Conclusions.** Plerixafor is safe and effective as a first line mobilisation agent and yields superior $CD34^+$ cell yields than historical chemotherapy based schedules. Patients mobilised by first line plerixafor have an excellent probability of proceeding uneventfully from harvest to transplantation, with acceptable engraftment time and 12-month outcome.

1121

INDICATIONS FOR HIGH-DOSE THERAPY WITH AUTOLOGOUS STEM CELL RESCUE AND FOR ALLOGENEIC TRANSPLANTATION IN PATIENTS WITH FOLLICULAR LYMPHOMA: A CONSENSUS PROJECT OF THE EBMT LYMPHOMA WORKING PARTY (LWP)

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Background. Despite the significant improvement in the outcome of patients with follicular lymphoma (FL) in recent years, FL is still incurable with conventional treatment. Defining the role of high-dose therapy with autologous stem cell rescue (HDT-ASCR) and of allogeneic transplantation in the therapeutic algorithm of FL is a major challenge, given the lack of informative prospective trials in the rituximab era. **Methods.** A panel of international experts on transplant/lymphoma were invited to join the project. The members of the panel decided in advance the statements to discuss and agreed the threshold to define consensus. Nineteen statements were subsequently scored individually and anonymously by each participant using a 9-point scale (1-3: disagree; 4-6: neither agree nor disagree; 7-9: agree). After discarding the highest and lowest ratings, consensus was reached if all the scores fell in the same group. No consensus was reached if there was >1 ratings in group 1-3 and >1 in group 7-9. Consensus was assessed after two rounds of rating, according to the threshold previously defined. Results. Consensus was reached on the following statements: 1) HDT-ASCR is not an appropriate treatment option to consolidate first remission in patients responding to immuno-chemotherapy, outside the setting of clinical trials; 2) In patients in first relapse with chemo-sensitive disease HDT-ASCR is an appropriate treatment option to consolidate remission; 3) Remission consolidation with HDT-ASCR is an appropriate treatment option in first relapse in patients with a short response duration (<3 years) after immuno-chemotherapy; 4) Remission consolidation with HDT-ASCR is an appropriate treatment option in first relapse in patients with high-risk FLIPI at relapse; 5) Remission consolidation with HDT-ASCR is an appropriate treatment option in patients in second or subsequent relapses with chemosensitive disease; 6) Allotransplant should be considered in patients with relapse after HDT-ASCR; 7) Reduced-intensity/non-myeloablative conditioning regimens are generally more appropriate in patients receiving an allotransplant; 8) In FL, the available biological and genetic risk factors are not sufficient to guide treatment decisions (including the indication for HDT-ASCR and allotransplant), but they are mainly guided by the clinical course. In addition, there was consensus to reject that HDT-ASCR is an appropriate treatment option to consolidate first remission in patients with high-risk FLIPI or grade 3a FL at diagnosis. However, no consensus could be reached about the role of HDT-ASCR in low-risk first relapse (low-risk FLIPI, long first remission or rituximab-naïve) or the criteria to select patients in whom allotransplant should be preferred over a first HDT-ASCR. Conclusions: Despite the undoubted impact of rituximab on the outcome of patients with FL, HDT-ASCR remains a strong treatment option at first relapse, especially in patients with high-risk features (i.e short response duration, high-risk FLIPI). In contrast, no specific indications for allotransplant (other than at relapse following HDT-ASCR) were agreed. This project demonstrates that, in the absence of evidence-based data, consensus methods may provide valuable tools to define indications for hematopoietic stem cell transplantation in FL.

Adult acute lymphoblastic leukemia - Clinical

1122

ANTI-CD19 BITE BLINATUMOMAB INDUCES HIGH COMPLETE REMISSION RATE AND PROLONGS OVERALL SURVIVAL IN ADULT PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Relapsed/refractory B-precursor ALL in adults has a dismal prognosis where only 35-40% of patients reach a hematological complete remission (CR) with a median overall survival of approximately 4.5 months. An exploratory phase II trial was conducted in this patient cohort with blinatumomab, a bispecific T-cell engaging (BiTE®) antibody that directs cytotoxic T-cells to CD19 expressing target cells.The primary endpoint was hematological CR or CR with partial hematological recovery (CRh*) within 2 cycles of blinatumomab. Secondary endpoints included overall survival and safety.Blinatumomab was administered by continuous intravenous infusion for 28 days followed by a 14-day treatment-free interval. Responding patients could receive 3 additional cycles of treatment or proceed to allogeneic hematopoietic stem cell transplantation (HSCT). Three dose levels were explored as shown in Table 1.In total, 36 patients were enrolled; data of 25 are currently available. Seventeen out of 25 treated patients (68%) achieved a hematological CR/CRh*. Five out of 17 responders (29%) had a CRh*.

Table 1. Summary of Dose Cohorts and Outcomes (Oct 2011).

Cohort	Patients Treated	Week 1, Cycle 1 µg/m²/day	Week 2, Cycle 1 µg/m²/day	Weeks 3-4, Cycle 1 and Subsequent Cycles µg/m²/day	CR/CRh*
1	7	15	15	15	5
2a (Final dose)	5	5	15	15	4
2b	6	5	15	30	3
3 (Final dose)	7	5	15	15	5
Total	25				17

All responders reached a minimal residual disease level below 10^{-4} within the first 2 cycles. Six patients proceeded to HSCT in CR/CRh* after blinatumomab treatment, and one of them developed a medullary $CD19^-$ relapse after HSCT. Eleven patients did not receive HSCT. Five of them relapsed: 2 relapses were $CD19^-$ (1 medullary and 1 extramedullary) and 3 were $CD19^+$ (1 medullary and 2 extramedullary).For the first 18 patients (cohort 1, 2a and 2b), median response duration is 7.1 months (218 days); median survival is not reached and the current median follow-up time for overall survival is 11.4 months.Cytokine release syndrome (CRS) and CNS events were reported as medically important events. In 2 patients with high tumor burden and no cytoreductive prephase, 3 CRS events were observed. Both patients received further blinatumomab therapy without CRS recurrence. Fully reversible adverse drug reactions of the CNS leading to treatment interruption were observed in 5 patients: 3 patients with seizures and 2 patients with disorientation. CNS symptoms fully resolved and all 5 patients were able to resume treatment at a lower dose.As final dose and schedule, 5 µg/m²/day in week 1 and 15 µg/m²/day for the remaining treatment (cohort 2a and 3) was selected for further investigation. In these cohorts (n=12), the most common treatment emergent adverse events (TEAEs, all grade 1-2) were pyrexia (67%), headache (33%) and tremor (33%). TEAEs of grade ≥3 (7 in 5 patients, no grade 4), irrespective of relationship, were infections, confusion, epilepsy, hypertension and thrombocytopenia.The final dosing regimen of blinatumomab produced an exceptionally high CR respectively MRD response rate and was well-tolerated. A global phase II study to confirm these data is underway.

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FIRST-LINE DASATINIB PLUS CONVENTIONAL CHEMOTHERAPY IN ADULTS WITH NEWLY DIAGNOSED PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA: THE KOREAN PROSPECTIVE PHASE II STUDY

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Background. Front-line combination of imatinib with conventional chemotherapy has demonstrated an improved complete response (CR) rate, an increased transplantation proceeding rate, and a better survival in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph-positive ALL). However, in the light of disease aggressiveness and recurrence, mainly as a result of the outgrowth of leukemic subclones with imatinib-resistant mutations, an improved strategy to induce more effective leukemic cell clearance is clearly needed. Dasatinib has been shown to be effective in patients with imatinib-resistant Ph-positive ALL. **Aims.** Here, we present the interim results of the Korean prospective phase II study protocol designed to evaluate the clinical efficacy of first-line dasatinib plus conventional chemotherapy for adults with newly diagnosed Ph-positive ALL. **Methods.** This study is registered at www.ClinicalTrials.gov as NCT01004497. The protocol enrolls patients (15-65 years) who receive dasatinib (100 mg once daily for 4 weeks) as an alternative schedule after each conventional chemotherapy course (alternating modified hyper-CVAD and high-dose cytarabine/mitoxantrone). Patients who have a suitable donor undergo allogeneic transplantation as early as possible. Patients without a donor continue to receive dasatinib plus conventional chemotherapy (up to 4 courses; depending on the patient's tolerability) followed by dasatinib maintenance therapy (100 mg/d for up to 2 years). Minimal residual disease monitoring for BCR-ABL1 transcript is centrally evaluated by real-time quantitative PCR (4.5 log sensitivity) through handling of bone marrow samples from all patients (Research Institute of Molecular Genetics, The Catholic University of Korea,Seoul,Korea). All patients in the study provided written informed consent, and the study protocol was approved by the institutional review board of The Catholic University of Korea. **Results.** Recruitment started in March 2010 and was completed in February 2012 (n=51). Of these, 8 patients are receiving the first or second dasatinib cycles (too early); 4 patients died from infections before starting dasatinib administration. Thus, 39 patients are evaluable for assessment of response. Median age was 42 (19-64) years. Karyotype analysis revealed additional chromosomal changes in 26 patients (67%). Twenty-seven patients (69%) had m-BCR transcript. All patients (100%) have achieved CR by the first dasatinib cycle, and of these, 18 patients (46%) have achieved major molecular response [MMR; including 7 complete molecular response ($CMR^{4.5}$)]. By the second dasatinib cycle, 30 patients (77%) have achieved MMR (including 17 $CMR^{4.5}$). No dasatinib-related serious adverse events (≥grade 3 toxicity) have been observed. To date, 30 patients (77%) have undergone allogeneic transplantation in CR. With a median follow-up duration of 14 months, 34 patients are alive and 31 are in continuous CR; 5 patients died in CR (2 infections during consolidation chemotherapy, 3 transplant-related com-

plications). Three patients have relapsed (CR durations were 4, 6, and 9 months, respectively) with T315I mutations. The 1-year cumulative incidence of relapse was 10%, and the 1-year disease-free survival and overall survival rates were 75% and 84%, respectively. **Conclusions.** Our data indicates that first-line combination of dasatinib with conventional chemotherapy appears to be effective in achieving a good quality of molecular response (MMR or CMR[4.5]) in adults with Ph-positive ALL.

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LONG TERM FOLLOW UP OF ELDERLY PATIENTS WITH PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA (PH+ALL): UPDATED RESULTS OF THE GMALL ELDERLY TRIALS

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Background. Incorporation of imatinib in front-line treatment strategies for Ph+ALL results in complete remission rates exceeding 90%, but long-term outcome remains unsatisfactory in patients not undergoing allogeneic stem cell transplantation (SCT). In a previously reported randomized trial of imatinib-based induction and consolidation chemotherapy in elderly patients with Ph+ALL, overall survival (OS) and disease-free survival (DFS) of all 55 patients at 24 months was 42% ± 8% and 19.2% ± 6.4%, respectively (Ottmann OG et al., Cancer. 2007; 109:2068-76). Published data on long-term outcome of elderly Ph+ALL patients, most of whom are not considered candidates for SCT, are very limited.**Aims.** To determine the proportion of long-term surviving elderly Ph+ALL patients receiving imatinib combined with intensive consolidation, examine the characteristics of the subset of long-term survivors, and obtain preliminary results on the feasibility and efficacy of SCT in this patient population. **Study design and patients demographics:** Our current analysis includes a total of 122 patients (120 ALL, 2 CML in lymphoid blast crisis), with a median age of 66 years (range 54-80). Fifty-five patients were enrolled in a previously reported randomized clinical trial comparing single-agent imatinib and chemotherapy as induction therapy, followed by up to 6 cycles of consolidation chemotherapy; a further 67 patients were subsequently treated according to this protocol as per recommendation by the GMALL Study Group.**Results.** The overall CR rate was 87%, median remission duration and OS was 16.6 months (range 1-79.4) and 18.3 months (range 0.5-80), respectively. Probabilities of remission duration, survival and DFS at 5 years were 8%, 18% and 8%, respectively. The type of initial induction therapy had no significant impact on OS and DFS. In univariate analysis, pulmonary disease was the only comorbidity associated with inferior outcome (median OS 10 months vs. 20 months, evaluable 59 pts.). Allogeneic SCT was performed in CR1 in 11 patients and as salvage therapy in 6 patients. Median age of these 18 patients was 62y (range 54-69). The time from diagnosis to SCT in CR1 was 133 days (87 d - 509 d) and from relapse to SCT in >CR1 92 days (63 d - 185 d).The 5yr OS in patients transplanted in CR1 vs. non-transplanted patients was superior (48% vs 13%). Remarkably, OS of the 7 patients transplanted beyond CR1 as part of salvage therapy was 43% after 3 years. With a median follow-up of 18.7 months (range 1.3 - 50) after SCT, 7 patients are in ongoing CR with a median OS of 34.7 mo. (range 16 - 50), 4 pts. died in CR, 7 pts. relapsed.**Conclusions:** The combination of imatinib in conjunction with intensive chemotherapy is feasible in elderly patients, but is associated with extremely poor survival primarily due to high relapse rate. Despite the small patient number, allogeneic SCT in CR1 is superior and should be considered as front-line therapy in this elderly patient population. Moreover, SCT as part of salvage therapy beyond CR1 is a feasible treatment option.

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MONITORING OF ASPARAGINE LEVEL AND ANTI-ASPARAGINASE ANTIBODY (AB) IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH THE GRAALL-2005 PROTOCOL

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L-asparaginase is a component of therapy for ALL. Asparagine depletion is considered as a surrogate marker for L-asparaginase activity (Wetzler, Blood 2007) but silent neutralizing anti-asparaginase Abs are associated with poor prognostic in children (Panosyan, J Pediatr Hematol Oncol 2004).We investigated asparagine depletion and Ab detection in adult patients treated in 5 GRAALL centers within the GRAALL-2005 study, derived from the published GRAALL-2003 protocol (Huguet, J Clin Oncol 2009). Asparagine level was evaluated by reversed-phase liquid chromatographic/tandem mass spectrometric method (Piraud, Rapid Commun Mass Spectrom 2005) while anti-asparaginase Abs were detected by ELISA (Wang, J Immunols **Methods.** 2000). After informed consent, 38 patients were included. Induction included administration of 6,000 IU/m² L-asparaginase (Kidrolase®) at d8, 10, 12 then at d20, 22, 24, 26 and 28, inorder to not interact with anthracycline, cyclophosphamide and vincristine. Before the first L-asparaginase infusion at d8, median asparagine basal level was 39 µmol/L (25-60), showing then full depletion (<2 µmol/L) at d13, 20 and 29 in97%, (28/29), 100% (30/30) and 100% (26/26) of patients who received the planned infusions, respectively. Anti-asparaginase Abs were detected (positive above 0.2) in 0% (26/26), 3% (1/29), 0% (29/29) and 4% (1/26) of patients at day 8, 13, 20 and 29, respectively. Two patients had low Ab levels (0.089 and 0.112) at d29, subsequently followed by a level increase in one patient and a clinical allergic reaction associated with depletion failure in the other, suggesting that Ab level as low as 0.1 may be clinically significant. At the onset of first consolidation block (d40 to d59), lack of depletion was observed in 32% (6/19) patients and Abs were detected in all of them, as well as in 2 patients still fully depleted but in whom consolidation was started before d35, leading to a 42% (8/19) Ab positivity at consolidation initiation. Interestingly, the first consolidation block was given earlier after d28 of induction in patients without Ab (median, 5 days) than patients with Ab (median, 12 days). L-asparaginase (10,000 UI/m²) was reintroduced during consolidation blocks 1, 2, 4 and 5. During these blocks, 48% of patients (11/23) demonstrated Ab positivity, 48% of patients (11/23) presented an anaphylactic reaction (10 with Ab, 1 without). Among the 4 patients with Ab tested for asparagine depletion at that time, 3 were not depleted (others pending). Conversely, among 6 patients without Ab tested, all were fully depleted. L-asparaginase was then reintroduced during late intensification and 8 patients were studied at that time (6 of them receiving 12,000 IU/m² Erwinase® instead of 6,000 IU/m² Kidrolase® due to earlier allergic reaction or CNS toxicity). All these 8 patients experienced full depletion.In conclusion, all patients were correctly depleted during induction, despite no L-asparaginase infusion between d12 and d20. During consolidation, 48% of patients had Ab and 48% anaphylactic reaction. Switch for Erwinase® allowed correct re-depletion during late intensification despite previous immunization. These results suggest that monitoring of asparagine depletion and anti-asparaginase Ab may be usefull to individualize L-asparaginase therapy in adults treated for ALL.

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MAIN RESULTS OF GRASPALL/GRAALL-SA2-2008 STUDY: L-ASPARAGINASE-LOADED RED BLOOD CELLS ADDED TO EWALL CHEMOTHERAPY IN OLDER PATIENTS WITH PHILADELPHIA CHROMOSOME-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Despite a widespread use in children and young adults, L-asparaginase has been limited in older patients with Philadelphia Chromosome-Negative (Ph-) ALL because of frequent and severe toxicities. With its improved efficacy/tolerability potential, L-asparaginase-loaded red blood cells (GRASPA®) could be a suitable option for combining L-asparaginase with standard chemotherapy in this population.**Aims.** The aim of GRASPALL/GRAALL-SA2-2008 study was to determine the optimal dose of GRASPA® that could be combined with the standard EWALL chemotherapy backbone in patients aged >55y with newly diagnosed Ph-ALL. Haematological, molecular Ig/TCR minimal residual disease (MRD) response and survival were the major secondary endpoints. **Methods.** It was an open label Phase II dose escalation study using a Bayesian experimental plan. Primary composite endpoint included tolerance and efficacy, the latter being defined as the achievement of target asparagine depletion for at least 7 days. Three doses of Graspa® (50, 100 and 150 IU/kg), infused at d3 and d6 of two successive induction cycles, were investigated. After each 3-pts cohort, an independent Data Safety Monitoring Board reviewed study drug tolerance before opening the next dose. EWALL backbone consisted of dexamethasone prephase followed by induction-1 (dexamethasone d1-2/8-11, vincristine d1,8, and idarubicine d1-2/8-9) and induction-2 (cyclophosphamide d15-17 and cytarabine d16-19/23-26). Consolidation consisted of 6 monthly alternating cycles with methotrexate (d1) / E.coli asparaginase (d2) and high-dose cytarabine (d1,3,5). Maintenance included mercaptopurine, methotrexate and vincristine/dexamethasone pulses for 2 years. Dose reductions were recommended for patients aged >70y.*Results.* Between March 2009 and October 2010, 30 patients were recruited in 20 centres in France. The 50, 100 and 150 dose levels included 3, 13 and 14 patients, respectively. Median age was 67 years (range 59-77). No differences in baseline characteristics were observed across the 3 dose level groups. Overall, 0 (0%), 2 (15%) and 5 (36%) patients presented limiting toxicities in the 50, 100 and 150 dose level, respectively. Because of insufficient serum asparagine depletion, only 3 patients received the lower dose. At the higher 100 and 150 dose levels, 85% and 71% patients reached target asparagine depletion, respectively. During induction, grade 3/4 infections were observed in 69% and 71% patients and invasive fungal infection in 23% and 43% patients, in the 100 and 150 IU/kg groups respectively. Anti L-asparaginase Abs were detected in 0/3, 2/13 and 1/14 patients after induction-1 and 1/2, 3/9 and 5/9 patients after induction-2, in the 50, 100 and 150 dose level group, respectively. Nevertheless, no clinical allergy was observed after the second drug infusion, but allergic reactions to native E-Coli L-asparaginase were observed in 5/19 pts after consolidation 1.Early response to therapy as well as event-free survival (EFS) and overall survival (OS) are presented in the table below according to the GRASPA dose.*Conclusion*: Two infusions of GRASPA® appears to be and active manner of introducing L-asparaginase during induction chemotherapy of older patients with Ph-ALL. The dose of 100 IU/kg showed the higher activity/safety profile so far but larger prospective evaluations of this new L-asparaginase formulation are warranted.

	Dose levels			
	50 IU/kg n=3	100 IU/kg n=13	150 IU/kg n=14	All
Induction failure	0/3 (0%)	1/13 (8%)	2/14 (14%)	3/30 (10%)
Induction death	1/3 (33%)	1/13 (8%)	4/14 (28%)	6/30 (20%)
Complete Remission at end of induction	2/3 (77%)	10/13 (77%)	9/14 (64%)	21/30 (70%)
MRD clearance at end of induction ($<10^{-3}$)	2/2 (100%)	6/8 (75%)	3/4 (75%)	11/14 (79%)

Dose	Median EFS	1-year EFS	2-year EFS	Median OS	1-year OS	2-year OS
100 IU/kg	11.8 mo	46%	23%	15.6 mo	62%	23%
150 IU/kg	4.0 mo	29%	na	9.5 mo	43%	na

Biology of poor-risk acute myeloid leukemia

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FLT3-ITDS INTRODUCE A MYELOID DIFFERENTIATION AND TRANSFORMATION BIAS IN LYMPHO-MYELOID MULTIPOTENT PROGENITORS

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Background. A striking but poorly understood feature of many oncogenic mutations is their lineage fidelity. Whilst this might simply reflect that mutations target a specific cell lineage, more intriguingly, if a mutation targets a primitive multipotent cell, it might impact on lineage fate decisions.**Aims.** To understand how oncogenic mutations might influence lineage specification using Flt3-ITDs as an informative model as, although wild-type FLT3 strongly promotes early lymphoid development, FLT3-ITDs are paradoxically restricted to myeloid-lineage leukaemias. **Methods.** We explored the impact of ITDs on leukaemic transformation and lineage capability of primitive multipotent haematopoietic cells using a Flt3-ITD knock-in mouse model to better understand the cellular and molecular basis for this myeloid-bias.**Results.** To determine the impact of FLT3-ITDs on leukaemic transformation, we generated Flt3-ITDRunx1$^{fl/fl}$Mx1-Cre^{+} mice. Importantly, Runx1 loss-of-function mutations occur in patients with both myeloid and lymphoid lineage acute leukaemias. Unexpectedly, even without poly I:C induction, Flt3-ITDRunx1$^{fl/fl}$Mx1-Cre^{+} mice developed a Runx1-deleted, high-penetrance, short-latency acute leukaemia of universal myeloid phenotype. This model recapitulates a particularly poor prognostic form of AML by targeting two collaborating mutations to their endogenous genetic loci which individually induce a relatively mild phenotype. This also demonstrates a key role for Flt3-ITD signaling in introducing a myeloid transformation bias.Flt3-ITD mice showed a cell-extrinsic and marked suppression of hematopoietic stem cells. The most primitive population expanded by Flt3-ITDs were LSKCD48+150- multipotent progenitors (MPPs), comprising >90% of LSK cells. Nanofluidic and global gene-expression analysis demonstrated that Flt3-ITD MPPs were myeloid-primed with loss of megakaryocyte and erythroid (MkE) priming. Lymphoid transcriptional priming, present at high-level in wild-type MPPs, was downregulated in Flt3-ITD MPPs. These findings were confirmed in functional assays through absent MkE and reduced lymphoid potential of Flt3-ITD MPPs, and together demonstrate that primitive lympho-myeloid MPPs, a population recently implicated in human AML, are expanded and biased towards myeloid development by Flt3-ITDs.In agreement with reduced lymphoid-priming of Flt3-ITD MPPs, analysis of early thymic development demonstrated a 10-fold reduction of early thymic progenitors (DN1 Kit+) as well as suppression of later stages of thymic development in Flt3-ITD mice. Expression of the chemokine receptor Ccr9 was reduced in Flt3-ITD MPPs, suggesting a suppression of thymic seeding by Flt3-ITDs. Furthermore, pre-pro-B cells, which retain both B-cell and myeloid potential, were markedly expanded and myeloid-bias in Flt3-ITD mice, whereas subsequent stages of committed CD19+ B-cells were reduced.To explore the mechanistic basis for this ITD-induced myeloid-bias, we examined levels of Pu1, a master-regulator of myeloid commitment during early hematopoiesis, using a Pu1-YFP reporter. Expression of Pu1 was significantly increased in LSK cells (1.4 fold) and in pre-pro-B cells (2.6 fold) in Flt3-ITD mice. Moreover, gene-set enrichment analysis in MPPs demonstrated upregulation of Pu1 target genes in Flt3-ITD mice. Furthermore, the loss of mature B-cells in Flt3-ITD mice could be partially rescued by Pu1 haploinsufficiency.**Summary:** These findings demonstrate how activating growth factor receptor mutations might subvert lineage commitment pathways and decisively influence the lineage outcome of the resulting cancer already at the pre-commitment stage.

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A LEUKEMIA STEM CELL GENE EXPRESSION SIGNATURE ASSOCIATES WITH A DISTINCT MICRORNA EXPRESSION PROFILE AND WORSE OUTCOMES IN OLDER ADULTS WITH PRIMARY CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA

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Background. Acute myeloid leukemia (AML) is hypothesized to be sustained by self-renewing leukemia stem cells (LSCs). Recently, Eppert et al (Nat.Med.2011;17:1086-93) studied functionally defined AML LSC populations, derived an LSC-specific gene expression signature (GES), and compared it with a normal hematopoietic stem cell (HSC) signature. Expression of a "core enriched"(CE) GES, which was based on the LSC signature and represents genes commonly activated in both normal and leukemic stem cells, was linked to worse survival in CN-AML. However, whether other molecular markers and microRNA (miR) expression associate with and contribute to the prognostic impact of the CE GES remains unknown.**Aims.** To study associations of the CE GES with known molecular prognosticators, miR expression, and outcomes in cytogenetically normal (CN-)AML. **Methods.** Gene/miR expression profiling was performed on pretreatment marrow/blood samples from 200 older (60-83 years[y]) patients with primary CN-AML enrolled on frontline Cancer and Leukemia Group B protocols, using Affymetrix HG-U133plus2.0 and OSU-CCCv4 microarrays. The CE score, a linear combination of CE GES expression values, was derived as described by Eppert et al. CE-high and CE-low groups were divided at the median CE score of all patients. Patients were also characterized for *NPM1,WT1,CEBPA,IDH1/IDH2,TET2,DNMT3A,ASXL1&RUNX1* mutations, *FLT3*-internal tandem duplications (ITD)&tyrosine kinase domain (TKD) mutations, *MLL*-partial tandem duplications, and *BAALC,ERG&MN1* expression. **Results.** Compared with CE-low patients, CE-high patients had higher marrow blast percentages (*P*=.004), were more likely to have *FLT3*-ITD (48% vs 15%, *P*<.001), mutated *RUNX1* (26% vs 7%, *P*<.001), high *BAALC* (67% vs 36%, *P*<.001) and high *ERG* expression (77% vs 30%, *P*<.001). On the other hand, CE-high patients were less likely to belong to the European LeukemiaNet Favorable Genetic Group (*P*<.001), have *FLT3*-TKD (*P*=.01) and mutated *TET2* (*P*=.01).Compared with CE-low patients, CE-high patients showed significant upregulation of 21 miRs, with no miRs being downregulated. Some of the upregulated miRs are known to be highly expressed in HSCs, including *miR-125a/b* (enhance proliferation/disturb differentiation of myeloid progenitors), *miR-126/miR-126** (increase survival/inhibit apoptosis of AML blasts, downregulated in *NPM1*-mutated CN-AML), *miR-130a/b*, *miR-146*, *miR-155* (upregulated in *FLT3*-ITD-positive AML), and *miR-196a*.Regarding treatment outcomes, CE-high patients had a lower CR rate (58% vs 75%; *P*=.02), shorter disease-free survival (DFS; *P*<.001; 3y rate, 5% vs 27%), and shorter overall survival (OS; *P*<.001; 3y rate, 11% vs 28%) than CE-low patients. In multivariable analyses, CE-high status remained associated with shorter DFS (*P*<.001) after adjustment for R882-*DNMT3A* mutation status (*P*<.001) and *BAALC* expression (*P*=.02), and shorter OS (*P*<.001) after adjustment for R882-*DNMT3A* mutation status (*P*=.03), *BAALC* expression (*P*<.001), and WBC (*P*=.01). **Summary.** Expression of the CE GES, representing genes highly expressed in LSCs and HSCs, associates with known unfavorable molecular markers (*FLT3*-ITD, mutated *RUNX1* and high *BAALC/ERG* expression) in older CN-AML, but remains independently associated with inferior outcomes after adjustment for known prognosticators. Strong expression of the CE signature was paralleled by a characteristic miR signature, including upregulation of miRs with known importance for stem cell function. Our results suggest these miRs may be functionally important in LSCs and might thus be targets for novel therapies.

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ALTERED MIRNA AND GENE EXPRESSION IN ACUTE MYELOID LEUKEMIA WITH COMPLEX KARYOTYPE IDENTIFY NETWORKS OF PROGNOSTIC RELEVANCE

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Background. In acute myeloid leukemia with complex karyotype (CK-AML) *TP53* alterations are the most common known molecular lesions, and recently a link between p53 and miR-34a network has been identified to play an important role in the pathogenesis of many tumors. **Aims.** To further explore the p53-miR-34a axis in CK-AML we analyzed a large cohort with and without *TP53* alteration (n=57 *TP53*^{altered} and n=31 *TP53*^{unaltered} cases; alteration refers to either loss and/or mutation of *TP53*).Results. First, we profiled microRNA (miRNA) expression to delineate *TP53* alteration-associated miRNA profiles. This identified miR-34a and miR-100 as the most significantly down- and up-regulated miRNAs, respectively. Profiling gene expression we also found a distinct miR-34a expression-linked gene expression profile enriched for genes belonging to p53-associated pathways and implicated in cell cycle progression or apoptosis (such as the Biocarta pathways "Cell Cycle G2/M Checkpoint", "Cell Cycle G1/S Checkpoint", "Regulation of cell cycle progression by Plk3", or "Tumor Suppressor Arf pathway"). CK-AML with low miR-34a-expression (below median expression of the entire cohort, CK$^+$/miR-34a$^{low\ expression}$) were older (median age, 59 vs. 52 years, p=.006) and showed in trend higher LDH serum levels (median U/l, 591 vs. 415, p=.05). Subgroup analysis comparing cases with the lowest miR-34a expression (1st quartile, CK$^+$/miR-34a$^{1st\ quartile}$) with all other cases (2nd-4th quartiles, CK$^+$/miR-34a$^{2nd\text{-}4th\ quartiles}$) revealed higher WBC for CK$^+$/miR-34a$^{1st\ quartile}$ (p=.02). Furthermore, miR-34a-expression was associated with resistance to chemotherapy. Response to induction therapy for CK$^+$/miR-34a$^{1st\ quartile}$ and CK$^+$/miR-34a$^{2nd\text{-}4th\ quartiles}$ was as follows: complete remission (CR) 14% (3/21) vs. 42% (26/62) (p=.03), and refractory disease (RD) 57% (12/21) vs. 34% (21/62) (p=.07), respectively. Other variables predicting for poor response to induction therapy were age [CR 18% (6/34) vs. 47% (23/49) for CK-AML >60 years and CK-AML <60 years, respectively; p=.009] and *TP53* alteration [CR 25% (13/52) vs. 52% (16/31) for *TP53*^{altered} and *TP53*^{unaltered} CK-AML, respectively; p=.02]. MiR-34a-expression was associated with inferior survival, the 3-year estimated survival rates for CK$^+$/miR-34a$^{low\ expression}$ and CK$^+$/miR-34a$^{high\ expression}$ patients were as follows: overall survival (OS) 4% vs. 20% (p=.006), and event free survival (EFS) 0% vs. 7% (p=.003), respectively. Other variables predicting for inferior OS in univariable analysis were *TP53*^{altered} (p=.0007) and in trend age (>60 years; p=.05). Multivariable analysis revealed miR-34a-expression (HR, 1.47; 95%-CI, 1.06 to 2.03; p=.02), *TP53*^{altered} (HR, 2.93; 95%-CI, 1.43 to 6.02; p=.003), logarithm of WBC (HR, 1.71; 95%-CI 1.02 to 2.89; p=.04), and age (HR, 1.03; 95%-CI, 1.01 to 1.04, p=.008), but not LDH serum level or monosomy 7 as significant variables for OS. Of note, we also observed several cases with high miR-34a-expression despite a biallelic alteration of *TP53*. Explorative subgroup analysis revealed that among *TP53*$^{biallelic\ altered}$ CK-AML, those with high miR-34a-expression [4th quartile (n=11) compared to the other cases (n=30) of the *TP53*$^{biallelic\ altered}$ cohort] had a significantly better OS (p=.02). Summary and Conclusions. Detailed molecular profiling links impaired p53 to decreased miR-34a-expression but also suggests p53-independent mechanisms of miR-34a-induction. An improved understanding of this mechanism might provide novel therapeutic options to restore miR-34a-function and thereby induce cell cycle arrest and apoptosis in *TP53*^{altered} CK-AML.

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DEREGULATED EXPRESSION OF EVI1 DEFINES A POOR PROGNOSTIC SUBSET OF MLL-REARRANGED ACUTE MYELOID LEUKEMIAS: A STUDY OF THE GERMAN-AUSTRIAN AMLSG AND DUTCH-BELGIAN HOVON GROUPS

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Background. AML with balanced rearrangements of the *mixed-lineage leukemia* gene (*MLL*) on chromosome band 11q23 occur in ~5% of adult cases. Outcome of t(11q23) AML is reported to be poor, except for AMLs with t(9;11) (intermediate prognosis). Risk stratification still largely depends on clinical risk factors and the translocation type present. High expression of *ecotropic viral integration-1* (*EVI1+*) was shown to be associated with t(11q23) AML; furthermore, *EVI1+* appeared to confer inferior prognosis in this AML subset.**Aims.** We hypothesize that *EVI1+* may be one of the important genetic events underlying the clinical heterogeneity of t(11q23) AML and thus aimed to explore the prognostic impact of *EVI1+* in a large cohort of t(11q23) AMLs in the context of clinical features and genetic markers.*Patients and* **Methods.** 286 (3.6%) AMLs with t(11q23) were identified in a combined cohort of 7728 AML patients enrolled in German-Austrian AMLSG and Dutch-Belgian HOVON prospective treatment trials. Patients were enrolled in seven different trials of the AMLSG (HD93, HD98A, HD98B, 07-04, 06-04, SHG95, SHG99) and five trials of the HOVON/SAKK AML study groups (HO04/A, -29, -42/A, -43, -92). Material could be retrieved in 177 cases for *EVI1* expression analysis.*Results.* Most frequent t(11q23) AML subgroups were as follows: t(9;11)(p22;q23) (n=128, 44.8%), t(6;11)(q27;q23) (n=42, 14.7%), and t(*v*;11q23) (n=116, 40.5%). Most common translocation partners within t(*v*;11q23) were t(11;19)(q23;p13.1)/*MLL-ELL* (n=18, 15.5%) and t(10;11)(p13;q23)/*MLL-MLLT10* (n=14, 12.1%). *EVI1+* was found in 45.8% (81/177) of all t(11q23) cases, with t(6;11) showing the highest frequency (83.9%), followed by t(9;11) (40.0%), and t(*v*;11q23) (34.8%). Multivariable analysis among all available t(11q23) AMLs revealed *EVI1+* to be the sole prognostic factor, predicting for inferior overall (OS; *p*=.001, HR 2.30), relapse-free (RFS; *p*=.004, HR 2.19), and event-free survival (EFS; *p*=.004, HR 1.93). There was no difference in achievement of complete remission (CR) between *EVI1+* and *EVI1*$^-$ t(11q23) AMLs. Relapse probability of t(11q23) AMLs was significantly higher in the presence of simultaneous *EVI1* overexpression (cumulative incidence of relapse at 5 years 74.8% of *EVI1+ vs* 48.3% of *EVI1-*, *p*=.0002). *EVI1+* t(11q23) AMLs in first CR had a significantly better outcome after allogeneic transplantation compared with other consolidation therapies (5-year OS: 54.7% *vs* 0%; Mantel-Byar, *p*=.0006). Within the subgroup of t(9;11) AML, *EVI1*$^-$ AMLs had lower white blood cell counts (*p*=.01), higher platelet counts (*p*=.01), more commonly FAB M5 morphology, and frequently had additional trisomy 8 (39.6%; *p*<.001). Concurrent gene mutations were virtually absent in t(9;11) AMLs; only rare *FLT3*-ITDs were identified (5/89 cases; 5.6%), whereas no sequence variations were found for all other genes analyzed (*CEBPA*, *NPM1*, *RUNX1*, *IDH1/2*, *ASXL1*, *TET2*, *DNMT3A*). Following multivariable analyses among t(9;11) AMLs, *EVI1+* again was the sole independent adverse prognostic factor for OS (*p*=.004, HR 3.20), RFS (*p*=.002, HR 3.73), and EFS (*p*=.001, HR 3.27).*Summary:* High expression of *EVI1* is found in approximately 50% of all t(11q23) AMLs. *EVI1+* is the strongest adverse prognostic factor in all t(11q23) AMLs and in the subset of t(9;11) AMLs. Allogeneic SCT in first CR seems to be beneficial for patients with *EVI1+ MLL*-rearranged AML.

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LEUKEMIC STEM CELL FREQUENCY WITHIN MINIMAL RESIDUAL DISEASE PROVIDES ADDITIONAL PROGNOSTIC INFORMATION IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Relapses of acute myeloid leukemia (AML) after chemotherapy occur in about 40% of AML patients due to outgrowth of minimal residual disease (MRD) cells, which in turn originate from leukemic stem cells (LSC). Retrospective studies have shown that MRD frequency can predict clinical outcome. We have recently demonstrated the prognostic impact of MRD in a prospective study (*Terwijn, submitted),* emphasizing the importance of MRD assessment for future clinical decision making. However, relapses are still seen in patients with low MRD levels, indicating that these patients have remaining, but poorly detectable or undetectable disease. A possible explanation is that apart from the frequency of leukemic blast cells, also the much lower frequencies of LSC determine the chance for relapse. **Aims.** To assess whether in AML LSC frequencies offer prognostic information additional to MRD. **Methods.** LSCs, similar to normal hematopoietic stem cells (HSC), reside within the CD34+CD38- compartment. LSCs can be discriminated from HSC by aberrant expression of CLL-1 *(van Rhenen, Blood 2007)* and lineage markers *(van Rhenen, Leukemia 2007)*. However, these markers were often not, or only partly expressed on LSC, leaving marker negative LSC unidentified. In part of these cases secondary gating strategies like forward scatter (cell size) and sideward scatter (cell granularity) could be applied to discriminate LSCs from HSCs *(Terwijn, ASH 2010:759*). **Results.** LSC assessment using marker expression was possible in 179/250 (72%) CD34+ AML patients. Using a marker/secondary gating approach this percentage increased to 88% (219/250) and we have demonstrated for the first time the presumed importance of LSCs by showing that high LSC frequency in remission bone marrow predicts relapse in AML patients *(Terwijn, ASH 2010;759).*Since both MRD and LSC frequency have prognostic impact, we combined both in a series of 68 AML patients and found that LSC frequency assessment in remission bone marrow revealed a poor prognostic subgroup (n=12), with a median relapse-free survival of 12 months, within the patient group with low/undetectable MRD levels (n=49), thereby strongly improving the prognostic impact. Poor performing patients can now be defined as having high MRD, high LSC or both high MRD and LSC. To make LSC assessment possible in all CD34+ AML patients we are currently using a multiple marker approach, combined with the secondary parameters. Upon using multiple markers the number of patients potentially eligible for LSC assessment increased from 72% to 89%. Current efforts are also directed towards identification of LSCs in CD34 negative AML, with the ultimate aim to track LSC in all AML cases. In ongoing studies we will establish whether the prognostic impact of LSC frequency as well as the combination of LSC and MRD assessment holds in a prospective setting. If so, we think it will become important to include both MRD and LSC frequency assessment in clinical decision making. **Conclusions:** 1. LSC frequency together with MRD frequency best predicts adverse outcome in AML patients. 2. With a multiple marker/secondary parameter flow cytometry approach we are able to identify LSCs in almost 90% of the CD34+ AML patients.

Acute leukemia; signalling and relapse

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GENE MUTATION PATTERNS AND THEIR PROGNOSTIC SIGNIFICANCE IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA: RESULTS FROM THE PROSPECTIVE RANDOMIZED ACUTE LEUKEMIA FRENCH ASSOCIATION (ALFA) 0701 TRIAL

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Background. Acute myeloid leukemia (AML) is a biologically and clinically heterogeneous disease. Although karyotype remains the most powerful prognostic factor of AML, gene mutations have become increasingly important for prognostic assessment and therapeutic management of AML patients, especially in cytogenetically normal (CN) AML. Recently, we showed that the addition of fractionated doses of gemtuzumab ozogamicin (GO) to standard chemotherapy significantly improves outcome in older AML patients (Castaigne *et al.*, ASH meeting 2011).**Aims.** To evaluate the incidence of mutations in 8 genes, their associated characteristics and prognostic relevance in AML patients enrolled on the Acute Leukemia French Association (ALFA) 0701 trial. **Methods.** We studied a cohort of 278 patients (50-70 years) with primary AML treated in the ALFA-0701 trial. Patients were randomized to receive "3+7"induction without (arm A) or with (arm B) GO. Patients achieving CR/CRp received two "1+4"consolidation courses ± GO according to randomization arm. Diagnostic samples were analyzed by standard PCR-based methods and Sanger sequencing for the presence of *FLT3* internal tandem duplication (*FLT3*-ITD), *MLL* partial tandem duplication (*MLL*-PTD), *FLT3*-D835/I836, *NPM1, CEBPA, WT1 (*exons 7 and 9*), IDH1*R132*, IDH2*R140*, IDH2*R172*,* and *DNMT3A* (exons 8-9 and 11-23) mutations. The screening for *IDH1/2* and *DNMT3A* mutations was restricted to CN-AML.*Results.* In the whole patient cohort, incidence of *NPM1, FLT3*-ITD*, CEBPA, FLT3*-D835/I836, *WT1* and *MLL*-PTD mutations was 34%, 18%, 7%, 4%, 2.5% and 2%, respectively. Within the CN-AML subgroup (n=142), incidence of *NPM1, DNMT3A, FLT3*-ITD*, IDH1*R132, *CEBPA, IDH2*R140, *IDH2*R172, *MLL*-PTD, *WT1* and *FLT3*-D835/I836 mutations was 50%, 31%, 25%, 12%, 9%, 8%, 4.5%, 3%, 2.5% and 2%, respectively. *NPM1*, *FLT3*-ITD, *DNMT3A* and *CEBPA* mutations were associated with leukocytosis and *IDH2*R172 with leukopenia. *FLT3*-ITD, *IDH2*R140 and *DNMT3A* mutations were strongly associated with the presence of *NPM1* mutations. *FLT3*-ITD frequently co-occurred with *DNMT3A* but rarely with *IDH2*R140 mutations. Prognostic significance of mutations was investigated in the whole CN-AML cohort. In univariable analyses, *CEBPA* mutations predicted longer event-free survival (EFS) (P=0.039) but not overall survival (OS) (P=0.33). Longer but not significantly different EFS (P=0.089) and OS (P=0.092) were observed in case of *IDH2*R140 mutation. In contrast, *DNMT3A* mutations and, to a lesser extent *FLT3*-ITD, predicted shorter EFS (P=0.005 and 0.13, respectively) and OS (P=0.0010 and 0.035, respectively). Age, white blood cell count and other mutations studied, including *NPM1* mutations, did not significantly affect outcome. In multivariable analysis, *IDH2*R140 and *DNMT3A* mutations remained significantly predictive of EFS (P=0.05 and 0.01, respectively), while only *DNMT3A* mutational status was retained as significantly associated with OS (P=0.001). We next performed survival analysis in CN-AML considering each randomization arm separately. Notably, this analysis revealed that *FLT3*-ITD was associated with shorter EFS (P=0.023) and OS (P=0.0025) in arm A, but had no impact on EFS (P=0.90) and OS (P=0.71) in arm B.*Conclusions.* In this trial*,* the only prognostic factors identified in CN-AML were *IDH2*R140 and *DNMT3A* mutations, associated with favorable and adverse outcome, respectively. Importantly, our results suggest that the addition of fractionated doses of GO to standard chemotherapy could overcome the negative prognostic impact of *FLT3*-ITD.

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GENOME WIDE GENE EXPRESSION PROFILING OF PRIMARY AND RELAPSED PEDIATRIC AML SAMPLES REVEALS IMMUNE REGULATORY PATHWAYS AND EPIGENETIC FACTORS INVOLVED IN RELAPSE DEVELOPMENT

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Background. Due to optimization of induction treatment, approximately 90% of AML pediatric patients achieve a complete remission in high income countries. However, relapses still occur in 30-40% of patients and outcome in these patients is dismal. Prevention of relapse offers the best opportunity for improvement of outcome, however mechanisms involved in AML relapse development are largely unknown. **Aims.** By genome wide gene expression profiling, we aim to identify key molecules and signaling pathways that are involved in the development of relapses that may offer opportunities for targeted treatment. **Methods.** Total mRNA of primary and relapse bone marrow samples (>80% blasts, n=46) was obtained from 23 pediatric AML patients and used for Affymetrix HGU 133 plus 2.0 microarrays. Mutation analyses for relevant type I/II aberrations were performed in both primary and relapse samples. For statistical analyses we used BioConductor packages, Significance Analysis of Microarrays (SAM) and BRB Arraytools. Pathway analysis was performed using Ingenuity pathway analysis. **Results.** Analysis of paired diagnosis and relapse samples revealed large inter-patient differences in the number of genes that were differentially expressed. Unsupervised cluster analysis showed that for 61% of patients the primary leukemia and relapse sample cluster next to each other. The remaining paired samples clustered more distant and interestingly the majority of these pairs also showed changes in mutation status of *FLT3*, *RAS* and *WT1*. In concordance with previous results (Bachas *et al,* Blood, 2010), these events were associated with shorter time to relapse. Pathway analysis of matched primary AML and relapse samples of the differentially expressed genes (> 2 fold) revealed molecular pathways implicated in cancer, inflammatory disease, hematopoietic development and genetic disorders. Subset expression of previously published *FLT3/ITD*, *WT1* and *RAS* mutation specific signatures in individual patients, changed according to gain or loss of mutations. In patients without mutational shifts, the signatures retained their expression levels.By *in silico* class prediction, we found 31 genes to be differentially expressed and discriminative for diagnosis or relapse samples with an accuracy of 70%-83%, depending on the method. Of the 31 genes, 29 showed a lowered expression in the relapse sample when compared to the initial diagnosis sample (median intensity ratio diagnosis/relapse= 1.74, range=1.47-2.33, $P_{29th\ gene}$=0.032) and 7 of these 29 genes have functions in the maintenance of chromatin structure, among which a number of histone variants. Preliminary RT-PCR results on histone variants *HIST1H1C* and *HIST1H2BG* confirmed this for an independent set of patient samples. *In vitro* cytotoxicity experiments indicate that 4 AML cell lines show a strong sensitivity towards an experimental drug that specifically targets histone variant mediated apoptosis. Additional tests using primary patient material are currently performed.ConclusionsWe identified genes and pathways that were significantly differentially expressed between diagnosis and relapse samples. Mutational shifts contribute to changed gene expression. Depletion of specific histone variants may be an epigenetic phenomenon in relapse development and may be targetable via new experimental therapeutics. Our efforts are directed to determine if these findings will be instrumental to design therapies aimed at preventing relapses.

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CALCINEURIN IS REQUIRED FOR LEUKEMIA-INITIATING CELL ACTIVITY IN A MOUSE MODEL OF T-CELL LYMPHOBLASTIC LEUKEMIA

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Background. Engagement of cell surface receptors coupled to the activation of the phospholipase C leads to an increase in intracellular calcium that results in the activation of calcineurin (Cn), a serine/threonine phosphatase composed of a catalytic (CnA) and a regulatory (CnB) subunit. Calcium-dependent activation of Cn leads to dephosphorylation of its substrates, including the NFAT family of transcription factors (NFATc1-c4). Cn/NFAT signaling plays critical role in a number of developmental processes, but is best characterized for its implication in controlling many aspects of T cell biology. We have previously reported the sustained activation of Cn in human lymphoma, acute lymphoblastic leukemia (ALL) and mouse models of lymphoid leukemias/lymphomas. Preclinical studies showed that pharmacological targeting of Cn with cyclosporine A (CsA) and FK506 has clear anti-leukemic effects in T-ALL mouse models. Besides its inhibitory activity towards leukemic cells, systemic delivery of these compounds is likely to inhibit Cn in cells of the tumor microenvironment, possibly contributing to their anti-leukemic activity. **Aims**. To investigate the intrinsic function of calcineurin in leukemic cells, we generated mouse T-ALL in which Cn can be specifically inactivated in tumor cells without affecting the tumor microenvironment. **Methods.** We generated mouse models of human T-ALL induced by either an activated *Notch1* allele (ICN1) or the TEL-JAK2 fusion oncoprotein in which Cn inactivation is specifically obtained in leukemic cells by Cre-mediated deletion of a conditional, floxed *CnB1* allele. We examined consequences of Cn inactivation on leukemia dissemination and reinitiation in these models. **Results.** We found that Cn activation in leukemic cells is under stromal control and that Cn inactivation alters physical and functional interactions that leukemic cells establish with their microenvironment, including their abnormal adhesion, migration and defective leukemic cells survival and proliferation. We show that Cn favors but is not required for *in vivo* expansion of leukemic blasts. In contrast, Cn is critical for leukemia-initiating cell activity as analyzed in transplantation studies. Importantly, Cn ablation in *de novo* homed leukemic cells also abolished disease re-initiation. **Conclusions.** These results demonstrate that calcineurin has an intrinsic pro-oncogenic function in leukemic cells that affects several phenotypic traits of tumor cells that ultimately control leukemia dissemination and expansion *in vivo*. Besides, these findings indicate that calcineurin is a promising target to prevent T-ALL relapse and call for clinical trials combining conventional debulking therapies with- or followed by Cn inhibitors during consolidation therapy.

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EFFECTIVE TREATMENT OF HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA CHARACTERIZED BY HYPERACTIVATED MTOR IN A PRE-CLINICAL NOD/SCID/HUALL MOUSE MODEL

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Intensification of chemotherapy regimens have improved the outcome of patients suffering from acute lymphoblastic leukemia (ALL) presently achieving cure rates of above 80%. Still 20% of the patients encounter relapse associated with inferior outcome, especially if occurring early. Importantly, despite the efforts achieved by stratification strategies including detection of molecular minimal residual disease, the majority of relapse patients are initially not identified by current markers and stratified into non-high risk groups. This clearly emphasizes the need for additional prognostic markers that reflect features of leukemia biology and thereby point to novel therapeutic targets.Previously, we have described a strong association of leukemia cell engraftment of primary patient B cell precursor (BCP) ALL samples transplanted in a NOD/SCID/huALL mouse model and patient outcome. Rapid engraftment (time to leukemia, TTL^{short}) is indicative for early patient relapse and associated with a gene expression profile pointing to pathways involved in cell growth and proliferation, most prominently mammalian target of rapamycin (mTOR).In this study we now functionally address mTOR activation in xenograft ALL samples and evaluate inhibition of this key survival pathway as novel treatment strategy for ALL *ex vivo* and *in vivo*. Previously characterized xenografts with respect to their engraftment phenotype (TTL^{short}/early relapse, n=5 or TTL^{long}/no or late relapse, n=8) were re-transplanted onto NOD/SCID mice and ALL cells were harvested at disease onset from leukemia bearing mice. We analyzed mTOR pathway activation by flow cytometry assessing phosphorylation of ribosomal protein S6 (P-S6), a molecule downstream of mTOR. Increased P-S6 levels were detected in TTL^{short} compared to TTL^{long} xenografts (U-test, P= .019) implying constitutive mTOR hyperactivation in TTL^{short}/early relapse leukemia. Moreover, the mTOR inhibitor rapamycin and the dual PI3K/mTOR inhibitor BEZ235 were analyzed. Most interestingly, both inhibitors showed a significant reduction in pathway activation in the TTL^{short} but not in most of the TTL^{long} xenografts indicating that the hyperactivated mTOR pathway of this high risk ALL subtype can be efficiently targeted.We then investigated the effect of mTOR inhibition on individual patient derived ALL *in vivo*. TTL^{short} (n=3) or TTL^{long} (n=2) leukemia samples were transplanted onto NOD/SCID mice. Upon appearance of leukemia in the peripheral blood, mice were treated with rapamycin or solvent and time to reoccurrence (TTR) of leukemia was analyzed as time from treatment until onset of leukemia related morbidity. Most interestingly, mTOR inhibition led to a significant delay of post-treatment ALL reoccurrence in all TTL^{short}/high risk ALL xenografts in contrast to similar leukemia free survival of TTL^{long}/low risk leukemias irrespective of rapamycin treatment. In a clinical setting high risk disease is unlikely to be treated by one compound alone. Therefore, we combined mTOR inhibition with multidrug chemotherapy resembling ALL induction treatment and observed a significantly prolonged TTR in 2 TTL^{short} leukemias compared to chemotherapy alone.In summary, we show that TTL^{short}/early relapse leukemia is functionally characterized by hyperactivated mTOR signaling and can effectively be targeted by mTOR inhibition *ex vivo* and *in vivo* thus providing a novel therapeutic strategy for the treatment of high risk ALL.

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RAD001: A CLINICO-BIOLOGICAL PHASE I GOELAMS TRIAL OF EVEROLIMUS ASSOCIATION WITH HIGH DOSE CHEMOTHERAPY IN LATE RELAPSING AML PATIENTS UNDER 65 YEARS OF AGE

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Background. MTORC1 signaling pathway is constitutively activated in most AML. The RAD001 (Everolimus) is a specific allosteric inhibitor of mTORC1 whose effectiveness has been demonstrated in solid tumors. We present here the results of a Phase Ib trial combining RAD001 and intensive chemotherapy in patients under 65 years with AML in first relapse (NCT 01074086).Materials and methods:Patients (pts) under 65 years in relapse more than one year following their first CR after chemotherapy + / - auto or allograft were enrolled between March 2008 and December 2011. The treatment consisted of increasing doses of RAD001 from 10mg to 70mg, with 10mg increments, administered orally on day 1 and day 7 of chemotherapy (Daunorubicin 60mg/m2/D1-D3+aracytine 200mg/m2/D1-D7). A second course of induction chemotherapy was re-administered without RAD001 if medullary blasts> 5% at D15. The plasma inhibitory activity (PIA) of the pts on the phosphorylation of p70S6K in MOLM14 cell line and the RAD001 AUC were determined.Results. Twenty five pts were enrolled in this trial. Their median age was 50.3 years (median: 20 to 65), sex ratio M / F: 1/1 (13/12); subtypes of AML: AML0 = 1, AML1 = 4, AML2 = 2, AML4 = 2, AML5 = 6, chemotherapy related AML = 4, with multilineage dysplasia = 2, CBF AML= 2. Cytogenetics: intermediate (n = 19) including 17 normal, good (n = 2), poor (n = 4). Treatment was well tolerated with less than 10% toxicity of all grade, mainly gastrointestinal liver and lung toxicity. The SAE were: appendicitis and mucormycosis at 10mg dose level (DL), a grade 4 skin toxicity with erythema and ulceration at 20mg DL, Fournier gangrene and Candida parapsilosis sepsis considered as DLT at 50mg DL which justified the inclusion of three additional patients at 50mg, one interstitial pneumonia with alveolar hemorrhage caused by infection at 70mg DL where there are currently 4 patients included. 17/25 pts were in CR (68%), of which 11 had a double induction. Eight pts have been allografted.The PIA was tested. We observed a strong inhibition of phosphorylation of p70S6K in all samples, 4 hours after administration of RAD001, regardless of the DL (mean decrease 93%). At 60 mg and 70 mg DL, significant inhibition was maintained through D3. On day 7 with 70 mg, the average inhibition was still around 40%.The median RAD001 AUC (1.82mg.h / l) was reached from 50mg DL. The CR rate of pts with AUC above the median was 90% vs 45% if AUC <1.82mg.h / l (p = 0.01) and this result remained significant after adjustment for karyotype, Flt3 status and age.Conclusion:In this Phase Ib trial testing the combination of RAD001 and chemotherapy, the DLT is not reached at 70mg/week. The overall CR rate is 68%. Above 50 mg DL, the CR rate is 90%. The PIA observed at 70 mg DL of RAD001 shows sustained inhibition of mTORC1 activity in vivo. An expansion cohort is underway at 70mg DL and the promising CR rates should be confirmed.

MDS and AML - Clinical & translational

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SEQUENTIAL GAIN OF MUTATIONS IN SEVERE CONGENITAL NEUTROPENIA PROGRESSING TO ACUTE MYELOID LEUKEMIA

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Background. Severe congenital neutropenia (SCN) is a bone marrow failure syndrome frequently associated with constitutional mutations in neutrophil elastase *(ELANE)* and a high risk to progress towards acute myeloid leukemia (AML). SCN patients are routinely treated with colony-stimulating factor 3 (CSF3). Acquisition of truncating mutations in the granulocyte-colony stimulating factor receptor (*CSF3R*) gene is a known phenomenon in SCN patients; these mutations are found in approximately 80% of SCN patient who have progressed towards AML, while they can be present in a minority of bone marrow cells already years before leukemic progression. Hence, alterations other than *CSF3R* truncating mutations are involved in SCN evolution to AML. So far, these underlying mechanisms remain largely unknown. **Aims.** We aimed to identify commonly affected pathways in SCN and its progression towards AML by next-generation sequencing of sequential hematopoietic samples of SCN and SCN/AML patients. **Methods.** A 27-year old male with SCN (index patient), with an *ELANE* and a *CSF3R* truncating mutation, developed AML after 17 years of CSF3 treatment. Serial blood/marrow samples of this patient representing evolutionary stages of the disease, the first obtained 15 years before the AML became overt, were analysed. Acquired mutations in the leukemic blasts of this patient were determined using whole exome sequencing, followed by amplicon-based sequencing to detect them in earlier disease stages. As a germ line control, bone marrow derived fibroblasts were used. Single myeloid colony analysis was done to investigate co-occurrence of mutations in single myeloid progenitors early during the disease. The functional consequences of a new *CSF3R* mutation were analyzed in myeloid colony assays. **Results.** In the leukemic blasts of the index patient, we identified 12 acquired non-synonymous mutations in 11 genes. Three of these, a *CSF3R* truncating mutation and mutations in *LLGL2* and *ZC3H18*, were present already 15 years before the AML became overt and co-occurred in single myeloid progenitors already in the early SCN phase. The progenitor population carrying these mutations expanded in time, whereas clones solely harboring *CSF3R* mutations disappeared from the bone marrow. The other 9 mutations were only apparent in the AML phase and affected known AML-associated genes *(RUNX1* and *ASXL1*) and chromatin remodelers (*SUZ12* and *EP300)*. We designed a custom capture library, covering the 11 genes identified in the index case, their interaction partners and close homologues (32 genes in total) to identify mutations in an additional cohort of SCN (n=21) and SCN/AML (n=5) patients. These experiments are currently ongoing. Furthermore, a new extracellular *CSF3R* mutation was found in the leukemic blasts of the index patient, which was located on the allele that already carried the *CSF3R* truncating mutation. Strikingly, this new mutation induced autonomous proliferation of myeloid progenitors. **Conclusions**. Progression from SCN towards AML is a multistep process with mutations arising early during the neutropenia and late mutations appearing in the AML phase. A novel *CSF3R* mutation, imposing CSF3 independence on an already functionally defective *CSF3R* mutant, supports the involvement of abnormal CSF3 signaling in leukemic transformation of SCN.

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EFFICACY AND SAFETY OF ELTROMBOPAG FOR THE TREATMENT OF THROMBOCYTOPENIA OF LOW AND INT-1 RISK MDS: PRELIMINARY RESULTS OF A PROSPECTIVE, RANDOMIZED, SINGLE-BLIND PLACEBO-CONTROLLED TRIAL

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Background. Patients with severe thrombocytopenia due to myelodysplastic syndromes (MDS) are at a risk of bleeding. Lower IPSS risk patients receive platelet (PLT) transfusions, mainly in the presence of bleeding. Short therapeutic effect and the development of refractoriness to PLT transfusions motivate research in novel treatments. Eltrombopag is an orally bioavailable thrombopoietin receptor (TPO-R) agonist indicated for the treatment of thrombocytopenia in chronic immune idiopathic thrombocytopenia with insufficient response to corticosteroids, immunoglobulins, or splenectomy. **Aims.** We present preliminary results of a multicenter, prospective, randomized, single blind placebo-controlled trial to evaluate the safety and efficacy of eltrombopag in low and intermediate-1 risk MDS with PLT count $<30\times10^9$/L. Patients are ineligible for or relapsed or refractory to other treatments and naive to TPO-R agonists. **Methods.** Eltrombopag vs placebo (2:1 ratio) will be administered to 69 patients at a 50 mg daily initial dose with 50 mg increases every 2 weeks to a target PLT count of $100x10^9$/L. Dose interruptions or reductions are required for PLT $>200x10^9$/L or adverse events. PLT response is defined as response (R) if either an increase of PLT$\geq30\times10^9$/L from $\geq20\times10^9$/L or an increase to $>20\times10^9$/L from $<20\times10^9$/L and by at least 100% not due to transfusion is observed in the absence of bleeding; complete response (CR) if PLT $\geq100\times10^9$/L. Quality of life (QoL) is assessed from baseline with the QOL-E and EORTC QLQ-C30 questionnaires. **Results.** 12/69 patients (8 on active drug - Arm A) of median age 68, interquartile range 49-78, are enrolled and 2 are in screening at the time of the present report. Baseline median PLT count was 16×10^9/L (IQR 11-22$\times10^9$/L). Two cases (Arm A) had a WHO bleeding score >1. Only 1 patient (Arm A) had a bone marrow (BM) blast count >5%. In Arm A, four cases obtained an R and one a CR after 7 days and 3 of 6 evaluable patients had a CR after 14 days at the initial 50 mg daily dose; an additional patient had a CR at 6 weeks at a 250 mg dose. In Arm A no bleeding events occurred; at week 8 there was an increase in median PLT count from 16×10^9 to 120×10^9/L (IQR 51.5-189 10^9/L), while no change was observed with placebo (arm B), in which 9 bleeding events occurred. Among the 5 patients in Arm A reaching day 22 in study, QoL scores improved in Treatment Outcome Index in 4 cases; Physical, Functional, Fatigue, and Cognitive Well-being in 3; Social, and MDS-Specific dimensions in 2; and Emotional functioning in 1 case. Adverse events occurred in 4 patients in Arm A: grades 2 (n=2), 3 (n=1) and 4 (n=1), all unrelated to eltrombopag. One patient experienced a second adverse event (asthenia, grade 1) with a possible association with eltrombopag. Neither peripheral nor BM blast counts increased in either arms at interim analysis. **Conclusions.** Preliminary data suggest that Eltrombopag is effective and safe in the prevention of bleeding and in raising PLT counts in lower risk MDS patients with severe thrombocytopenia.

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CLINICAL FEATURES, TREATMENT AND PROGNOSIS IN PATIENTS WITH MONOSOMY 7-INTERIM RESULTS FROM A GERMAN MULTICENTER STUDY

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Background. Monosomy 7 is observed in about 2% of patients with myelodysplastic syndromes (MDS). Recent publications demonstrated a different prognostic impact for partial (del7q) as compared to total (-7) monosomy 7 (Cordoba et al., 2012, Schanz et al., 2012) and a therapeutic benefit for demethylating agents (DMA) in this group of patients (Fenaux et al., 2009). It was our aim to analyze the clinical features, prognosis and therapeutic strategies in a large, multicentric cohort study. **Patients and Methods.** To date, 172 patients with monosomy 7 were collected and retrospectively analyzed. Patient data was coalesced from the Universities of Göttingen (n=15; 9%), Düsseldorf (n=114; 66%), Freiburg (n=32; 19%) and Dresden (n=11; 6%). The median age of patients was 65 years, 65% were males. Based on the IPSS, 14.7% were classified as intermediate-1, 42.1% as intermediate-2 and 43.2% as high-risk. Concerning treatment, patients were classified as followed: Best supportive care (BSC), low-dose Chemotherapy (LDC), high-dose chemotherapy (HDC), demethylating agents (DMA; either 5-azacytidine or decitabine) and others. Patients who received allogeneic stem cell transplantation (allo-Tx) as the first line therapy were excluded from survival analyses. Those who were transplanted at a later stage were censored at the time of transplantation. **Results.** Patients with a monosomy 7 showed a higher proportion of advanced MDS stages at the time of the first diagnosis. In a recently published (Schanz et al., 2012) MDS study, RAEB-1/2 stages were found in 13.1% of all patients while in the present cohort, it was observed in 42.6% (p<0.01). The median and range was 9.1 g/dl (2.5-14.5) for hemoglobin (Hb), $1.3*10^3$/ml (0-22.4) for ANC and $75.0*10^3$/ml (3.0-843.0) for platelets, respectively. As compared to the independent cohort mentioned above, the median Hb did not show a pronounced difference (9.6 g/dl in the independent versus 9.1 g/dl in the -7/7q- cohort), while the median ANC ($2.1*10^3$/ml versus $1.3*10^3$/ml) and platelet count ($124*10^3$/ml versus $75*10^3$/ml) was clearly lower. The median survival in untreated patients with isolated -7 (n=21) was 12.5 months and 33.9 months (n=19) in del 7q (p=0.068); the median time to AML transformation was not reached in del(7q), while in -7 it was 14.4 months (p=0.149). The majority of patients were treated with best supportive care (BSC; 62.2%). The remaining patients received 1-3 sequential therapies (1: 23.8%; 2: 7.6%; 3: 6.4%). As a first line therapy, 21 patients (12.2%) received an allo-Tx, 6 (3.5%) HDC, 4 (2.3%) LDC, 23 (13.4%) DMA and 11 (6.4%) were treated with other therapies. Patients who received DMA at any course of their disease showed a median survival of 23.6 months as compared to 10.0 months in patients treated with any other therapy (p=0.025). The AML-free survival was 22.8 months in DMA vs. 14.4 in others (p not significant). **Conclusions.** Patients with monosomy 7 show distinct clinical features, prognostic impact and response to therapy. Here, we present first interim results of the German monosomy 7 cohort. Further data of the ongoing study will be presented in detail.The study is supported by research funding from Celgene

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HIGH LEVELS OF GLOBAL DNA METHYLATION ARE AN INDEPENDENT ADVERSE PROGNOSTIC FACTOR IN A SERIES OF 90 PATIENTS WITH DE NOVO MYELODYSPLASTIC SYNDROME (MDS)

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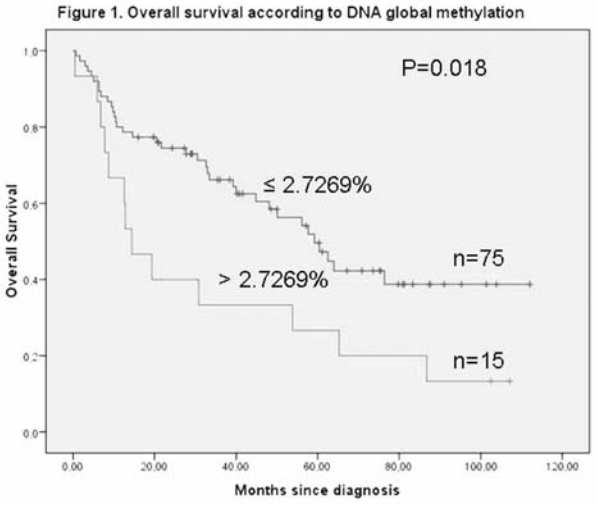
Background. Since the introduction of hypomethylating agents, the pathogenic involvement of methylation in the progression and prognosis of MDS has become of great interest. The DNA methyltransferase inhibitors have been the only treatment capable of significantly prolonging survival in high-risk MDS by inducing direct citotoxicity and global hypomethylation. The hydroxymethylation, which consists in adding a hydroxyl group at the 5' end of the 5-methylcytosines (5-mC), has been described as a passive DNA demethylation process inducing gene reexpression. **Aims.** To test whether 1) higher levels of global methylation are associated with a worse prognosis, and 2) higher hydroxymethylation levels predict a better outcome. **Methods.** Ninety patients (49M/41F; median age 75 years, range 32-97) with de novo MDS diagnosed from 2002 to 2009 were classified according to WHO 2008 criteria as RA (n=2), RARS (n=3), isolated del(5q) (n=1), RCMD (n=63), RAEB-1 (n=9), RAEB-2 (n=11), MDS-U (n=1). At the study closing date, median follow-up for surviving patients was 4.8 years (range 1.3 - 9.3 years). According to IPSS 77 patients belonged to the low risk (low, Int-1) categories and 13 patients to the high risk (Int-2, high) categories. We also applied the WPSS (65 low vs. 25 high) and R-IPSS (77 low vs. 13 high). Genomic DNA was isolated from bone marrow samples obtained at diagnosis using a commercial kit (Qiagen®). Global methylation and hydroxymethylation were determined in all patients by ELISA (Epigentek®) using anti-5-mC and anti-5-hydroxymethylcytosine (anti-5-hmC) monoclonal antibodies obtaining the percentage of 5-mC and 5-hydroxymethylcytosine (5-hmC) in total DNA. **Results.** Using the R software version 2.9.0. (maxstat), we estimated a significant cutpoint of the percentage of 5-mC: 2.7269%, near the 75 percentile, selecting 15 patients with global 5-mC levels above the cutpoint. We could not obtain an optimal cutpoint for the 5-hmC percentage with statistical significance. 5-hmC levels had no impact in overall survival (OS) or leukemia-free survival (LFS), moreover, we could not correlate it with IPSS, WPSS or R-IPSS risk subgroups. In contrast, patients with 5-mC below 2.7269% had a better OS than those with more methylated DNA (median 59.2 vs. 14.4 months; p=0.018; Figure 1). On the other hand, we observed a positive trend in terms of LFS (p=0.084). Furthermore, we observed higher 5-mC percentage at the high risk groups (poor and very poor) of R-IPSS using the Student's t-test (mean 2.8% vs. 2.1%; p < 0.001; 95% CI 0.5-0.8). It is of note that patients with transfusion requirement had a worse OS (p=0.01) and a shorter LFS (p=0.048). Using the Cox regression analysis including age, sex, 5-mC above 2.7269%, transfusion requirement and IPSS (Int-2/High), all these variables were independently associated with worse outcome except sex.**Conclusions:** Degree of global DNA methylation appeared as an independent prognostic factor in MDS patients. These results, if validated in larger series of patients, would support using this assay as a new prognostic tool and might guide the use of hypomethylating agents in the treatment of MDS.

Figure 1. Overall survival according to DNA global methylation



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OVEREXPRESSION OF THE TRANSCRIPTION FACTOR C/EBPG, ABERRANTLY EXPRESSED IN A SUBSET OF ACUTE MYELOID LEUKEMIAS, RESULTS IN A NEUTROPHILIC DIFFERENTIATION ARREST

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C/EBPg and C/EBPa are transcription factors that belong to the family of CCAAT/enhancer-binding proteins (C/EBPs). Proper C/EBP levels and activity are necessary during hematopoiesis, and defects in these proteins have been related to the development of AML. We recently demonstrated that C/EBPg is highly upregulated in a specific subset of AML samples characterized by silencing of C/EBPa. Similarly, C/EBPg was upregulated in C/EBPa knockout murine hematopoietic stem/progenitor cells (LSK). Downregulation of C/EBPg expression in murine C/EBPa deficient LKS cells or in human AML samples with C/EBPa silencing was sufficient to completely restore granulocytic differentiation, as demonstrated by in vitro experiments and transplantations assays. Given these observations, in the present study we investigate (1) whether and how C/EBPa regulates C/EBPg expression, (2) whether high C/EBPg levels are sufficient to induce a granulocytic differentiation arrest and (3) the mechanism by which C/EBPg overexpression results in a differentiation block. First, we performed C/EBPa chromatin immunoprecipitation (ChIP on Chip) in a C/EBPa expressing cell line, and showed that C/EBPa binds to the C/EBPg promoter. To determine whether C/EBPa could negatively regulate C/EBPg we generated a human C/EBPg promoter luciferase reporter construct and performed luciferase assays. Cotranfection of this reporter with increasing amounts of a C/EBPa expression plasmid showed that C/EBPa was able to repress C/EBPg promoter activity in a dose-dependent manner. Mutation and deletion reporter constructs demonstrated that the C/EBPa binding sites within the C/EBPg promoter were not required for the C/EBPa mediated repression and that C/EBPa reduced C/EBPg transactivation by affecting E2F1 activity. Next, to study the contribution of C/EBPg to the pathogenesis of AML we overexpressed C/EBPg in an *in vitro* differentiation model and in murine bone marrow cells, and observed that sustained expression of this transcription factor induced a block of neutrophilic differentiation. Using a C/EBPg-ER inducible system, we demonstrated that C/EBPg regulates crucial granulocytic differentiation factors, such as C/EBPa and G-CSF-R. Altogether our experiments indicate that C/EBPa binds and represses C/EBPg expression, that enforced C/EBPg expression results in a granulocytic differentiation block and that this block is mediated by the repression of myeloid differentiation drivers. In conclusion our study provides novel insights of the contribution of C/EBPg to the development of AML in a specific subset of AML.

Multiple myeloma - Clinical

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LENALIDOMIDE MAINTENANCE SIGNIFICANTLY REDUCES THE RISK OF PROGRESSION IN NEWLY DIAGNOSED YOUNG MULTIPLE MYELOMA PATIENTS ENROLLED IN RV-MM-PI-209 TRIAL

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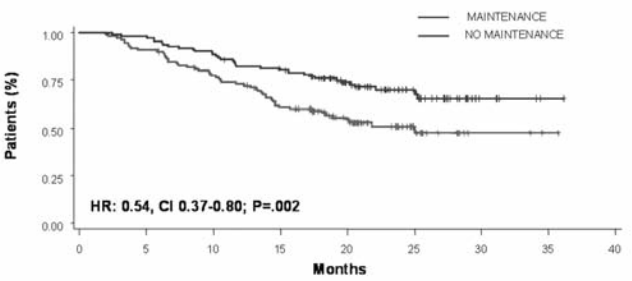
Background. High-dose therapy with autologous stem cell transplantation (ASCT) is considered the standard treatment for patients with multiple myeloma (MM) who are younger than 65 years. The incorporation of new drugs into induction, consolidation and maintenance therapy is changing the treatment paradigm of newly diagnosed MM.**Aims.** To compare in a prospective randomized trial (MM-RV-PI209) conventional chemotherapy plus novel agents [melphalan-prednisone plus lenalidomide (MPR)] with tandem high-dose melphalan (melphalan 200 mg/m2 with stem-cell support; MEL200), both followed by maintenance with lenalidomide or no maintenance.

Figure. Progression free-survival by maintenance



Methods. Four-hundred two patients were enrolled and received induction treatment with four 28-day cycles of lenalidomide (25 mg, days 1-21) in combination with low-dose dexamethasone (40 mg, days 1,8,15,22). After induction therapy, 202 patients were randomly allocated to MPR treatment [six 28-day cycles of melphalan (0.18 mg/kg days 1-4), prednisone (2 mg/kg days 1-4) and lenalidomide (10 mg days 1-21)] followed by maintenance with lenalidomide (10 mg, days 1-21; N=98) or no maintenance (N=104). The 200 other patients were assigned to receive MEL200, followed by maintenance with lenalidomide (10 mg, days 1-21; N=100) or no maintenance (N=100). Progression-free survival (PFS) was the primary endpoint of this study. Data were analyzed in intention-to-treat. **Results.** Patients characteristics were well balanced, including median age (58 years in both groups), ISS and FISH abnormalities [presence of t(4;14) or t(14;16) or del17p]. Response rates were similar after consolidation (MPR vs MEL200), with very good partial response (VGPR) or better of 60% vs. 58% (p=0.24) and complete response (CR) rate of 20% vs. 25% (p=0.49). After a median follow-up of 33 months (IQR 26-37), the 3-years PFS was 38% in MPR and 59% in MEL200 (HR=0.60, p=0.0009). The 3-year overall survival (OS) was similar: 82% with MPR and 85% with MEL200 (HR 0.87, p=0.57). The median duration of maintenance was 18.4 months (IQR 13-22). Lenalidomide maintenance did not significantly increase response rate: CR rate was 20% after MPR and 22% after maintenance, while it was 25% after MEL200 and 30% after maintenance. The 2-year PFS from the start of maintenance was 66% for patients randomized to maintenance and 47% for the no-maintenance arm, demonstrating that maintenance with lenalidomide reduced the risk of disease pro-

gression by 46% (HR 0.54, p=0.002, Figure). The 2-year OS survival was similar: 88% with lenalidomide maintenance and 85% without maintenance (HR 0.71, p=0.28). Patients who received MPR plus lenalidomide maintenance had a similar 2-year PFS compared with patients receiving MEL200 without maintenance (59% and 63%, respectively, p=NS). The rate of second primary malignancies was 2% in both maintenance arms. **Conclusions.** Lenalidomide maintenance significantly reduced the risk of progression in newly diagnosed young MM patients. PFS of patients receiving MPR plus maintenance is comparable to MEL200 without maintenance, at present no difference in survival is reported.

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DARATUMUMAB, A CD38 MONOCLONAL ANTIBODY IN PATIENTS WITH MULTIPLE MYELOMA - PRELIMINARY EFFICACY AND PHARMACOKINETICS DATA FROM A DOSE-ESCALATION PHASE I/II STUDY

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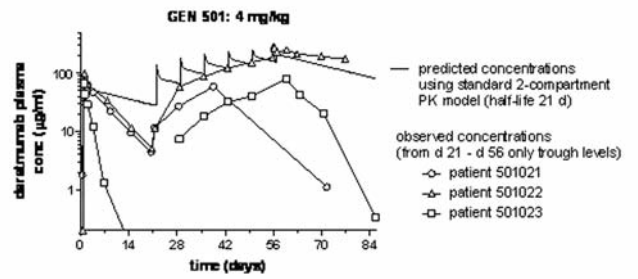
Background. Daratumumab (HuMax®-CD38) is a human CD38 monoclonal antibody with broad-spectrum killing activity; it effectively kills CD38-expressing tumour cells via antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and apoptosis. In this ongoing first-in-human (FIH) dose-escalation study of daratumumab in pts with multiple myeloma (MM) (ClinicalTrials.gov CT00574288), the safety profile has been accetable[1].AimThe purpose of this ongoing FIH study is to establish the safety profile and maximal tolerated dose. Furthermore, exploratory assessments of efficacy and pharmacokinetics (PK) will be performed.MethodsPts ≥18 years and previously diagnosed with MM requiring systemic therapy and considered relapsed or refractory to at least two different prior lines of therapy and not eligible for salvage ASCT were enrolled. The design of this study encompasses an accelerated dose-escalation based on a classical 3+3 design. Daratumumab is administrated over a 9 week period encompassing 2 pre-doses and 7 full-doses.



The doses range from 0.005 mg/kg to 24 mg/kg.Daratumumab plasma concentrations were measured by ELISA. Plasma levels were compared with those predicted using a standard 2-compartment PK model with V_{cen} = 40 ml/kg and elimination half life = 21 days.Evaluation of efficacy data was according to Rajkumar[2]. This abstract presents preliminary data on PK and efficacy, and the results presented are based on data analyzed before database lock.ResultsData from 23 pts including the 4 mg/kg cohort are collected.PK analysis showed plasma peak levels as expected, but relatively rapid and variable clearance at low dose levels. Clearance rate decreased with increasing dose, which suggests an effect of target binding on the PK. At 4 mg/kg, the impact of target-mediated clearance tended to become small and plasma concentrations went up towards the range expected for non-binding IgG (Figure).Preliminary efficacy evaluation in this abstract is based on best response to paraprotein as reflected by change in serum and/or urine M-component. For groups ≤ 1 mg/kg, 3/17 pts achieved a reduction in serum M-component (12%, 14%, 19%); in the 2 mg/kg group, 1/3 pts had a reduction in urine M-component (55%); and in the 4 mg/kg group, 3/3 pts had a reduction in the serum M-component of 49%, 55%, and 64%, respectively. Furthermore, a marked reduction to normal level in the percentage of bone marrow plasma cells was seen in the 4 mg/kg group in all patients, 80%, 89%, and 97%, respectively.The toxicity has been manageable.Conclusion-In pts with relapsed or refractory MM treated with daratumumab, a reduction in M-component and bone marrow plasma cells has been observed in the 4 mg/kg cohort. No unexpected accumulation of daratumumab has been seen in patients treated with 4mg/kg during 9 weeks of treatment. The toxicity has been manageable. Further data will be presented at the meeting.[1]Gimsing: ASH 2011 abstract 1873[2]Rajkumar: Blood 2011;117:4691-5

1144

MLN9708, AN INVESTIGATIONAL PROTEASOME INHIBITOR, COMBINED WITH LENALIDOMIDE AND DEXAMETHASONE IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA PATIENTS: EVALUATION OF WEEKLY AND TWICE-WEEKLY DOSING REGIMENS

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Background. MLN9708 is an oral, reversible, and specific 20S proteasome inhibitor under clinical evaluation in multiple tumor types, including previously untreated multiple myeloma (MM). The feasibility and efficacy of combining a proteasome inhibitor with an immunomodulatory drug and a steroid in previously untreated MM has been demonstrated with the bortezomib, lenalidomide, and dexamethasone (RVD) regimen.**Aims.** Two Phase 1/2 studies (NCT01217957, weekly MLN9708, and NCT01383928, twice-weekly MLN9708) are ongoing to determine the safety, maximum tolerated dose (MTD), recommended Phase 2 dose (RP2D), and anti-tumor activity of oral MLN9708 in combination with lenalidomide and dexamethasone.

	MLN9708 W (n=51)	MLN9708 TW (n=6)
MTD / RP2D in combination with lenalidomide and dexamethasone	2.97 mg/m² / 2.23 mg/m² (converted to a 4.0 mg fixed dose based on population PK results)	Not reached (escalated to 3.7 mg to date)
Drug-related AEs, n (%)	41 (80)	4 (67)
Most common drug-related AEs, n (%)		
Fatigue	14 (27)	1 (17)
Nausea	13 (25)	0
Vomiting	11 (22)	0
Thrombocytopenia	10 (20)	0
All-cause grade ≥3 AEs, n (%)	26 (51)	3 (50)
Drug-related grade ≥3 AEs, n (%)	19 (37)	1 (17)
Most common related grade ≥3 AEs, n (%)		
Diarrhea	2 (4)	0
Erythematous rash	2 (4)	0
Syncope	2 (4)	0
Vomiting	2 (4)	0
Serious AEs, n (%)	16 (31)	1 (17)
Discontinuations due to AEs, n (%)	2 (4)	0
On-study deaths, n	0	0

Methods. Patients with previously untreated MM aged ≥18 years with measurable disease received weekly (W) or twice-weekly (TW) oral MLN9708: W dosing, MLN9708 days 1, 8, and 15, lenalidomide 25 mg days 1-21, and dexamethasone 40 mg days 1, 8, 15, and 22 of 28-day cycles; TW dosing, MLN9708 on days 1, 4, 8, and 11, lenalidomide 25 mg days 1-14, and dexamethasone 20/10 mg (cycles 1-8/cycles 9-16) days 1, 2, 4, 5, 8, 9, 11, and 12 of 21-day cycles. Dose escalation proceeded using a standard 3+3 schema based on the occurrence of dose-limiting toxicities in cycle 1. Adverse events (AEs) were graded by NCI-CTCAE v4. Response was assessed by IMWG criteria with nCR included. **Results.** At data cut-off (Jan 27, 2012), 51 patients (median age 65 years [range 38-86]; 82% ISS stage I/II) had received MLN9708 W (15 Phase 1, 1.68-3.95 mg/m^2; 36 Phase 2, 4.0 mg fixed dose), and 6 patients (median age 64 years [range 56-78]; all ISS stage I/II) had received MLN9708 TW (all Phase 1, 4 at 3.0 mg, 2 at 3.7 mg). To date, on the W schedule, Phase 1 and 2 patients have received a median of 6 (range 1-13) and 2 (range 1-4) cycles, respectively; TW patients have received a median of 2.5 cycles (range 1-3). 39 W and all 6 TW patients remain on treatment; 6 W patients discontinued to receive ASCT. Safety data are shown in the Table. Drug-related grade ≥3 toxicity was reversible and included diarrhea, erythematous rash, syncope, and vomiting (each n=2) on the W schedule, and hyperglycemia (n=1) on the TW schedule. Any-grade drug-related peripheral neuropathy (PN) was observed in 7 patients on the W schedule and 1 patient on the TW schedule; there was only 1 patient with grade ≥3 PN on the W schedule (at 3.95 mg/m^2, a dose above the MTD). Of 38 response-evaluable W patients, 34 (89%) have achieved ≥PR, including 7 CR (1 sCR), 5 VGPR, and 22 PR; all remain in response with duration of response of up to 13 cycles. All 3 response-evaluable TW patients have achieved ≥PR, including 2 VGPR and 1 nCR after cycle 2. Response evaluation is ongoing.**Conclusions:**Data suggest that both W and TW oral MLN9708 plus lenalidomide and dexamethasone are generally well tolerated with manageable toxicity and demonstrate encouraging signs of anti-tumor activity in patients with previously untreated MM.

1145

A RANDOMIZED PHASE 2 STUDY OF ELOTUZUMAB WITH LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background. Elotuzumab is a humanized monoclonal IgG1 antibody targeting CS1, a cell surface glycoprotein. CS1 is highly expressed on >95% of multiple myeloma (MM) cells, with lower expression on natural killer cells and little to no expression on normal tissues. A phase 1 trial of elotuzumab plus lenalidomide and low-dose dexamethasone demonstrated an 82% objective response rate (ORR) in patients with relapsed/refractory (RR) MM (Lonial et al. J Clin Oncol, in press). **Aims.** To determine safety and efficacy of elotuzumab in combination with lenalidomide and low-dose dexamethasone in patients with RR MM including those with high-risk disease. **Methods.** In this phase 2 study, previously treated patients with MM were randomized to elotuzumab 10 or 20 mg/kg IV (days 1, 8, 15, and 22 every 28-days in first 2 cycles and days 1 and 15 of subsequent cycles), lenalidomide 25 mg PO (days 1-21) and dexamethasone 40 mg PO weekly. Prophylaxis for infusion-related reactions (IRs) was administered prior to each elotuzumab infusion. Treatment continued until disease progression or unacceptable toxicity. The primary objective was to assess efficacy (ORR ≥partial response [PR]) according to IMWG criteria.Results. Among 73 patients (median age 63 years; range, 39-82), 55% had received ≥2 prior therapies, 60% had received prior bortezomib, and 62% prior thalidomide. ORR was 82% for all patients including 48% ≥ very good PR (VGPR). The ORRs were 92% in the 10 mg/kg group (n=36) and 73% in the 20 mg/kg group (n=37). Median time to objective response was 1.0 months (range, 0.7-5.8). After a median follow-up of 14.1 months, median progression-free survival (PFS) has not been reached, with PFS rates of 75% (10 mg/kg) and 65% (20 mg/kg). ORRs of 80% and 81% were shown in patients with high-risk cytogenetics and/or Stage 2-3 MM, respectively. The most common grade 3/4 toxicities were neutropenia (16%), lymphopenia (16%), and thrombocytopenia (16%). Investigator-designated IRs were reported in 12% of patients (all grades); 1 patient (1.4%) had grade 3 IR (rash). Summary and Conclusions. Elotuzumab in combination with lenalidomide and low-dose dexamethasone was generally well tolerated and resulted in a high ORR, and PFS not reached after 14.1 months of median follow-up in patients with RR MM. The combination also showed encouraging activity in patients with high-risk cytogenetics and/or Stage 2-3 MM. Updated results will be presented at the meeting. Two phase 3 trials of 10 mg/kg elotuzumab in this combination are ongoing in newly diagnosed MM (ELOQUENT1; CA204-006; NCT01335399) and RR MM (ELOQUENT2; CA204-004; NCT01239797).

1146

A MUTLI-INSTITUTIONAL EXPERIENCE WITH UPFRONT CYCLOPHOSPHAMIDE, BORTEZOMIB AND DEXAMETHASONE (CVD) IN THE TREATMENT OF AL AMYLOIDOSIS

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Background. Bortezomib combinations have shown great promise in the treatment of AL amyloidosis. Here we present the largest multi-centre experience to date using the cyclophosphamide, bortezomib and dexamethasone (CVD) regimen as upfront therapy. The series is representative of patients from France, the USA, and the UK.**Objective.** Characterise the response to CVD when used in the upfront treatment of AL amyloidosis. **Patients and Methods.** The primary cohort was comprised of 75 patients treated between 2006-2011. Bortezomib (1.0-1.3 mg/m^2) was administered on either the a weekly (days 1, 8, 15 and 22 in a 5 week schedule) or twice weekly (days 1, 4, 8 and 11 in a 3 week schedulte) with oral dexamethasone (10 or 20 mg, 4 to 8 doses) and cyclophosphamide (300 mg/m^2 (max 500 mg) on days 1, 8 and 15). Complete information for staging by the Mayo clinic criteria was available in 73 patients, and 73% were stage III based on biomarkers obtained prior to the initiation of CVD. Haematologic and dFLC responses were measured and analyzed on an intention-to-treat (ITT) basis. Deaths during therapy were deemed non-responders. Overall survival (OS) was estimated from the start of treatment and measured in months (m). BNP response were also assessed as previously described. Data use was approved by the institutional review boards at each site. Written consent was obtained from all patients. **Results.** Median follow-up was 7.9m (0.0-37.3). Average number of cycles given was 4 (1-8). By ITT 74 patients were assessable for response based on conventional hematologic response criteria. Seventy one patients were assessable for a dFLC response. By ITT the overall hematologic response rate (RR) was 78% (CR = 42% and dFLC-VGPR = 54%). Eighteen patients (24%) died within 6 m of therapy all with stage III disease. Analyzing the patients assessable for response at 6 m of treatment the RR was 92%, CR rate was 51% and dFLC-VGPR rate was 63%. Mayo cardiac staging succesfully identified high risk patients (figure 1A). Thirty one patients were assessable for a NT-proBNP response which was acheived in 10 patients (35%). Median OS has not been reached. The estimated 1-year OS was 74%. Attaining both a CR and dFLC-VGPR correlated with a significant improvement in OS (figure 1B). Neuropathy was seen in 19% of patients but contributed to discontinuation of treatment in only 8%. **Conclusion:** This retrospective series lends further support to the use of bortezomib containing regimens in the treatment of AL amyloidosis, especially in the upfront setting. It is the largest series of patients treated with CVD reported to date. Despite a large proportion of patients with advanced Mayo stage disease high rates of CRs and dFLC-VGPRs are attained. In

addition, it emphasizes the importance of attaining deep clonal responses to maximize outcome. Overall, these response rates compare favorably to previously published treatment options for this disease and corroborate recently published single-institution experience with the CVD combination. Larger prospective phase III studies are warranted and are underway.

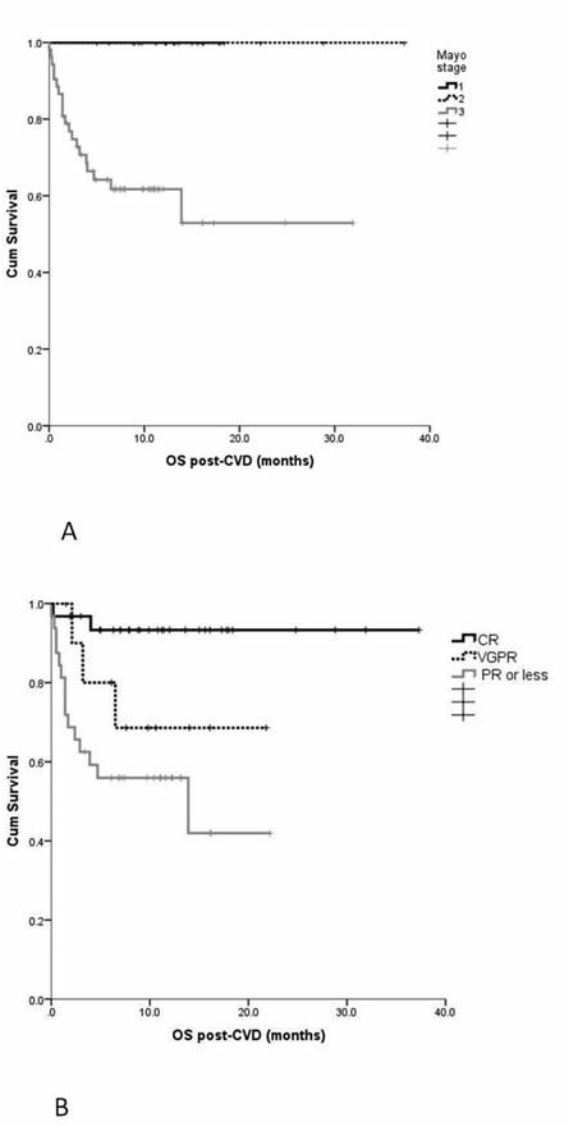

Figure 1. Overall survival in based on (A) Mayo cardiac stage (P=0.03) and (B) depth of hematologic response (P=0.001).

Stem cell biology

1147

VON WILLEBRAND FACTOR EXPRESSION IDENTIFIES A SUBPOPULATION OF THROMBOPOIETIN-DEPENDENT MEGAKARYOCYTIC-BIASED HEMATOPOIETIC STEM CELLS

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Primitive multipotent stem and progenitor cells express at low level lineage-specific genes otherwise restricted to mature blood cell types, an event known as lineage priming. Although, the concept of multi-lineage transcriptional priming is well established, its importance in lineage commitment is less understood. We observed that the megakaryocyte and endothelium restricted gene encoding von Willebrand factor (*Vwf*) is expressed at relatively high levels in phenotypically defined hematopoietic stem cells (HSCs). To determine if *Vwf* expression and megakaryocytic lineage priming identifies a novel subset of potentially megakaryocytic biased HSCs, we generated a bacterial artificial chromosome (BAC) transgenic reporter mouse line expressing enhanced green fluorescent protein (EGFP) under control of murine *Vwf* regulatory elements. Flow cytometry phenotyping of this reporter line showed that *Vwf*-EGFP transgene is selectively expressed in a fraction of phenotypic HSCs, in megakaryocyte progenitors and platelets. We fractionated the adult long term hematopoietic stem cell (LT-HSC: LSKCD150^{+}CD48^{-}CD34^{-}) compartment using *Vwf*-EGFP transgene, isolated and characterized molecular and functionally both subpopulations. Global gene profiling and gene set enrichment analysis demonstrated that *Vwf*-EGFP^{+} HSCs are megakaryocyte transcriptionally primed, expressing genes present in a megakaryocyte-specific molecular signature as determined by Q-PCR. When transplanted at low numbers, *Vwf*-EGFP^{+} HSCs are more efficient in producing platelets/myeloid cells than *Vwf*-EGFP^{-} HSCs whereas the latter, although fulfilling the HSC definition, give rise to more B and T cell progenitors. A hierarchy can be established between these two subpopulations, with *Vwf*-EGFP^{+} HSCs preceding *Vwf*-EGFP^{-} HSCs. To get insight into *Vwf*-EGFP^{+} HSCs regulation, we generated *Vwf*-EGFP$^{tg/+}$; *Thpo*$^{-/-}$ mice and observed that no *Vwf*-EGFP^{+} HSCs are present in these mice. Furthermore, thrombopoietin-deficient LSKCD34^{-}Flt3^{-} HSCs down-regulate megakaryocytic genes and up-regulate erythroid and myeloid genes in comparison to wild-type HSCs. These results suggest a role of thrombopoietin as a driver of the megakaryocytic bias observed in *Vwf*-EGFP^{+} HSCs. In summary, our work expand current knowledge on HSCs heterogeneity and reveal, to our knowledge, not previously described megakaryocytic-biased HSCs.

1148

STAT1-DEFICIENT MICE DEVELOP A MYELOPROLIFERATIVE SYNDROME RESEMBLING HUMAN CML

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Background. The transcription factor STAT1 (signal transducer and activator of transcription) is a member of the JAK-STAT signaling pathway and is activated by a wide range of cytokines and growth factors. Gene deletions in mice revealed the essential role for STAT1 in immunity and tumor surveillance. This can be attributed to an impaired Interferon (IFN) signaling, as well as to defective *Stat1-/-*cytotoxic T-cells (CTLs) and NK cells which are incapable of lysing tumor cells. Interestingly, several patients have been reported to suffer from germline STAT1 mutations or even from complete STAT1-deficiency, which is reflected by severe immunodeficiencies, especially with susceptibility to mycobacterial or viral diseases.Besides being a key factor in immune regulation, STAT1 is also considered an important tumor suppressor. We have recently shown that mice deficient for STAT1 develop mammary cancer, which can be attributed on the one hand to impaired CTL activity, on the other hand to enhanced proliferation of *Stat1-/-*mammary epithelial cells and their tendency to uncontrolled growth.Now we show for the first time that *Stat1-/-* mice spontaneously develop a myeloproliferative syndrome during age. **Aims.** The aim of the project is to characterize *Stat1-/-*leukemia and to understand the molecular mechanisms driving the disease. We further intend to investigate which cell is the origin of the disease and whether this cell also maintains leukemia. **Methods.** *& Results.* Analysis of *Stat1-/-*mice at different time points revealed, that increased amounts of Gr1^{+}Mac1^{+} myeloid cells can already be detected at an

early age in bone marrow and blood of *Stat1-/-*mice, compared to wild-type controls. In the course of time, these cells start to manifest as well in peripheral lymphoid organs like spleen and lymph nodes. Beginning at the age of 8 months *Stat1-/-*mice succumb to the disease - the animals suffer from severe hepatospleenomegaly and blood leukocyte counts reach numbers up to 300 000 per microliter. Bone marrow transplantation experiments revealed that the disease can be transferred into recipient animals.*Conclusion:Stat1-/-*mice develop a myeloproliferative syndrome, which can be transplanted into secondary recipient mice. The exact mechanism how the disease develops is still unclear and requires further investigation.

1149

ROLE OF JAM-B/JAM-C INTERACTION IN THE CIRCULATION OF HEMATOPOIETIC STEM CELLS AND THEIR RETENTION IN THE BONE MARROW

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Background. The junctional adhesion molecules JAM-B and JAM-C belong to the Ig superfamily and interact together. We have previously shown that JAM-C is involved in the regulation of the immune response and controls chemokine secretion by stromal cells in secondary lymphoid organs (Frontera, Arcangeli et al 2011). In adult mammals, cells from the blood system are continually generated by hematopoietic stem cells (HSC) that reside in the bone marrow (BM). HSC are maintained in a quiescent and undifferentiated state through adhesive interactions with specialized microenvironmental niches. We have recently demonstrated that JAM-B, expressed by BM stroma microenvironment, controls HSC maintenance and retention in the BM and delivers stemness signals to HSCs (Arcangeli et al Blood 2011). Indeed, *jam-b* deficient mice are characterized by increased BM cellularity and frequency of cycling HSCs, suggesting that HSC from *jam-b* deficient mice are more prone to exit the niche. This is consistent with our results showing, *jam-b* deficient mice exhibit an exacerbated response to mobilizing agents (Arcangeli et al Blood 2011).**Aims.** In the present study, we address the question whether JAM-B and JAM-C are involved in the circulation of hematopoietic progenitors in mouse as well as in human model of xenograft and whether targeting the JAM-B/JAM-C interaction can induce the mobilization of hematopoietic progenitors specifically.**Method:** Using flow cytometry, blocking antibodies, *jam-b* deficient mice and xenograft model in NGS mice, we show that the couple JAM-B/JAM-C controls the circulation of murine as well as human hematopoietic progenitors.**Results.** Targeting JAM-B/JAM-C interaction using anti-JAM-C blocking antibody induces specifically hematopoietic progenitor mobilization, but not mature cells. In addition, the anti-JAM-C blocking antibody does not mobilize progenitors of *jam-b* deficient mice showing that the anti-JAM-C dependent progenitor mobilization is dependent of JAM-B expression by BM stromal cells. Mobilized HSC have downregulated JAM-C expression at cell surface and have lost the ability to interact with its ligand, JAM-B. In addition, anti-JAM-C antibody prevents hematopoietic progenitor homing and engraftment in the BM suggesting that the couple JAM-B/JAM-C regulates the entrance as well as the adhesion of hematopoietic progenitors in specific niches (manuscript in preparation). In parallel, we show that human CD34+ hematopoietic progenitors express JAM-C and are able to use JAM-B to adhere *in vitro*. *In vitro*, the addition, in the culture medium, of JAM-B or JAM-C soluble proteins or anti-JAM-C blocking antibody alters the adhesion of CD34+ progenitor cells on MS5 stromal cell lines. Furthermore, *in vivo*, in xenograft models, we show that anti-JAM-C blocking antibody prevents CD34+ hematopoietic progenitor homing in murine BM suggesting that in humans, the couple JAM-B/JAM-C may also regulate the interaction between hematopoietic progenitor and their specific microenvironment (manuscript in preparation). **Summary:** Altogether, our data suggest that the interaction JAM-B/JAM-C participates to the crosstalk between the bone marrow microenvironment and the hematopoietic progenitors. Although the mechanism by which the couple JAM-B/JAM-C acts on this crosstalk remains unclear, evidences suggest that this couple acts in BM homeostasis by regulating chemokine secretion.

1150

THE TRANSCRIPTION FACTOR MEIS1 REGULATES COMMITMENT TOWARDS THE MEGAKARYOCYTE-ERYTHROCYTE LINEAGE BY REGULATING GATA1 EXPRESSION

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Background. The expression of specific transcription factors in hematopoietic stem and progenitor cells has been associated with the commitment towards a certain lineage fate.The homeobox transcription factor MEIS1 is required for hematopoiesis. Mice deficient for MEIS1 die at mid-gestation due to internal hemorrhages caused by defective vascularization and a lack of megakaryocytes and platelets. **Aims.** The aim of our studies is to unravel the contribution of MEIS1 to human hematopoiesis. **Methods.** MEIS1 knockdown and overexpression was achieved using lentiviral transductions. Transduced CD34+ human stem and progenitor cells were employed to semisolid media for colony formation. Cells were also harvested for RNA isolation and expression profiling.**Results.** Expression analysis of human hematopoietic stem and progenitor cell subpopulations showed that MEIS1 is present in the most immature hematopoietic stem cells (HSC: CD34+/CD38-) whereas expression decreased in more committed common myeloid progenitors (CMP: CD34+/CD38+/CD110-/CD45RA-), granulocyte-monocyte progenitors (GMP: CD34+/CD38+/CD110-/ CD45RA+) and megakaryocyte-erythrocyte-progenitors (MEP: CD34+/CD38+/CD110+/ CD45RA-). Lentiviral MEIS1 overexpression in CD34+ cells resulted in a 3-fold increase in erythroid colonies and a decrease in granulocyte-monocyte colonies. As total colony numbers were unaffected, the number of erythroid colonies increased at the expense of granulocyte-monocyte colonies. Concordantly, silencing MEIS1 abrogated erythroid colony formation. We therefore hypothesized that MEIS1 expression is important for the lineage decision of hematopoietic stem and progenitor cells towards a MEP fate. To elucidate at which level MEIS1 influences lineage fate, MEIS1 expression vectors were expressed in sorted CD34+ HSC, CMP, GMP and MEP. Erythroid colony formation increased 2-fold in HSC and 3-fold in the CMP subset indicating that MEIS1 is involved in the lineage decisions in these subsets. Interestingly, we also observed erythroid colony formation upon MEIS1 expression in sorted GMP indicating that MEIS1 overexpression can reprogram granulocyte-monocyte restricted progenitors into MEPs. Elevated MEIS1 expression in MEP does not affect colony composition compared to empty vector indeed indicating that lineage decision in less committed progenitors towards MEP is affected. We further investigated how MEIS1 exerts its effects. For this purpose we focused on the transcription factors GATA1 and PU.1. GATA1 upregulation negatively regulates PU.1 expression thereby restricting hematopoietic progenitor cells towards an erythroid fate. Vice versa, when PU.1 is induced cells are committed towards the granulocytic-monocytic lineage. Expression profiling of CD34+ cells 48 hours after transduction with MEIS1 expression vector showed increased GATA1 expression whereas PU.1 expression was not significantly altered. The CD34+ population in which we analysed Gata1 and PU.1 expression consisted mainly of CMP and GMP. GATA1 upregulation may be a first step to restrict the cells towards a MEP fate, which will be followed by PU.1 downregulation, subsequently.**In conclusion**, we show that MEIS1 is a crucial transcription factor for the megakaryocyte/erythroid lineage fate in human hematopoiesis and we provide evidence that GATA1 is a downstream target of MEIS1.

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NERVE GROWTH FACTOR AND COLLAGEN 1 PREVENT APOPTOSIS AND MAINTAIN DURABLE SELF-RENEWAL OF ADULT MOUSE HEMATOPOIETIC STEM CELLS STIMULATED WITH PROLIFERATIVE CYTOKINES

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Background. Self-renewal is a key property of hematopoietic stem cells (HSCs) and the hallmark of stem cells in general. However, despite decades of research, it is still unknown which extrinsic factors are necessary to optimize HSCs self-renewal *ex vivo*.**Aims.** The present experiments were designed to identify potential stromal-derived factors that promote mouse HSC self-renewal *ex vivo***. Methods.** Highly purified HSCs isolated from mouse adult bone marrow (i.e. $EPCR^+$, $CD150^+$, $CD48^-$, $CD45^+$ cells, 42% having longterm repopulating ability) were cultured for 7 days in vitro in serum-free medium (SFM) and various combinations of cytokines, stromal cells and factors they produce. HSCs numbers were determined by performing limiting dilution transplants in sub-lethally irradiated congenic *W41/W41* mice assessed for repopulation activity of the transplanted cells 4-6 months post-transplant. Culture conditions that supported the greatest expansion of HSCs were used to assess the HSC output of single input cells and to design comparative gene expression analyses on 6-hour-stimulated cells to identify potential stromal factors involved. These were then tested for their potential to replicate the effects of stroma cells and stromal cell conditioned medium.**Results.** Of the various conditions initially tested, the addition of stromal cells or stromal cell-conditioned medium (CM) to Steel factor and IL-11 gave maximal and equivalent HSC outputs after 7 days. (5- to 11-fold expansion of transplantable HSC numbers). This indicated a stromal cell contact-independent mechanism. Visual tracking of single-cell cultures revealed that stromal cell-derived factors prevented the apoptosis of ~50% of the input cells and transplant assays of the CM-containg cultures showed that 90% of the cells that produced at least one daughter HSC did so asymmetrically and only 10% did so symmetrically. Gene expression analysis showed that pathways activated by collagen 1 (Col1) and nerve growth factor (NGF) were significantly upregulated during the self-renewing process and a direct test of these two factors combined indicated they could replace the activity of CM in promoting HSC survival and expansion.**Conclusion:** NGF and Col1 are key stromal-derived factors that can positively regulate adult HSC expansion when these are stimulated with proliferative cytokines.

Targeted therapies

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BONE MARROW DERIVED MESENCHYMAL STROMAL CELL THERAPY FOR TREATING BIOLOGIC-REFRACTORY CROHN'S DISEASE, A MULTI-CENTRE AUSTRALIAN PHASE II STUDY

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Background. Mesenchymal stromal cells (MSCs) have immunomodulatory properties at multiple levels, involving action on T cells, B cells, dendritic and NK cells..These qualities have resulted in application to steroid-refractory acute graft versus host disease (AGVHD). Following a successful phase 1 trial in refractory GVHD (Herrmann, R et al, Int. J. Hem, 2012, 95 (2),182-128) we have expanded their application to biologic-refractory Crohn's disease and shortly will start a phase 2b randomised trial in de novo AGVHD. The treatment of advanced Crohn's disease has improved in recent years with the advent of the biologic therapies but in one third there is either primary non-response or a loss of response, resulting in the need for surgical removal of bowel. **Aims.** . The purpose of this study is to undertake an Australian multicentre phase II randomised safety and efficacy trial of allogeneic MSCs in biologic-refractory Crohn's disease where surgical intervention is declined. Four of eight Australian centres have obtained ethics approval for the study. The primary endpoint is clinical response (Crohn's disease activity index (CDAI) fall >100) and secondary endpoints include clinical remission (CDAI fall to <150) and endoscopic improvement at day 42. **Methods.** . MSCs are isolated after bone marrow aspiration from third party volunteer donors after informed consent and expanded under GMP in CTTWA, cryopreserved and assessed for microbial contamination as well as viability, phenotypic and cytogenetic characterisation. After patient screening, including mucosal biopsy for flow cytometric analysis and storage of samples for cytokine and chemokine array, patients receive four intravenous infusions of 2 x 10^6 MSCs per kg bodyweight, at weekly intervals.Results. To date 9 patients have been treated after giving informed consent; six have completed therapy, five (83%) demonstrating a clinical response (p=0.02) and three (50%) clinical remission to CDAI<150. Endoscopic improvement has been seen in two. All patients have experienced dysgeusia from the cryopreserving agent dimethyl sulfoxide. No infusion reactions, infections or secondary tumours have been seen to date.Logistic issues included the difficult (and often new) concept for ethics committees of cell therapy, the funding issues of an investigator led vs an industry study, the need for multi-disciplinary and multicentre collaboration and the ethical issues of obtaining normal volunteer donors.Conclusions. The study has proven feasible and regulatory and transport issues between states and capital cities overcome. Preliminary results are encouraging.

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CRIZOTINIB OBTAINS DURABLE RESPONSES IN ADVANCED CHEMORESISTANT ALK+ LYMPHOMA PATIENTS

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Background. The Anaplastic Lymphoma Kinase (ALK) gene is fused to several partner genes (mainly NMP) in the majority of Anaplastic Large Cell Lymphoma (ALCL) patients. This disease is characterized by deregulated ALK tyrosine kinase activity, through the phosphorylation of proteins involved in apoptosis, cellular proliferation and differentiation. Although ALK + ALCL are responsive to cytotoxic drugs, relapses occur and bear a poor prognosis.Crizotinib is an experimental, orally bio-available, ATP-competitive small-molecule inhibitor of c-Met/HGFR receptor tyrosine kinases with cellular IC50 values between 24 and 60 nM in NPM-ALK+ cells. The recommended dose in ALK + ALCL, previously established in Phase I study, is 250 mg BID.MethodsWe report the safety and efficacy results of crizotinib in 9 ALK+ lymphoma patients (age 20-56y), resistant to cytotoxic therapy who received crizotinib 250 mg BID as a part of compassionate use named patient protocol. Morphological diagnosis was ALCL in 7 cases and Diffuse Large Cell Lymphoma in 2 cases. All patients were resistant to at least 3 lines of treatment including autologous bone marrow transplant (ABMT) in 3 cases and allogenic bone marrow transplant (alloBMT) in one patient. All had involvment at multiple sites (nodal and extranodal) as well as B symptoms (fever and bone pain). No steroids or drugs with antineoplastic activity were permitted during crizotinib treatment. All ALK + diagnosis were confirmed by FISH analysis using an ALK break-apart probe. Disease status was also assessed by CT/PET scans following RECIST criteria.ResultsIn all cases of B symptoms disappeared (median time 5 days after start of crizotinib, range 2-10 days), and LDH levels normalized (median time 25 days, range 15-35 days).

Patient	Previous treatment	Age	ABMT y/n	Best response
1	CHOP DHAP HD-VP16 + ALL 2000 scheme	27	n	CR 20+
2	CHOP MAD	20	y	CR 2
3	BFM HD-CTX + ARA-C VD	23	y	PR 1
4	CHOP VCR-ARA C-Dx-PND HYPER-C-HYDAM	23	n	CR 15+
5	BEP CHOP ICE HD-MTX + HD ARAC HYPER-C_VAD RT	40	n	SD 1
6	CHOP DHAP	30	y	PR 1
7	IEV DHAP CHOP	66	n	CR 11+
8	CHOP DHAP Dexa-BEAM	28	n	PRO
9	CHOP, ESHAP, ESHAP	34	n (alloBMT)	CR 8+

BM aspirates were ALK-positive in 4 patients by FISH and became negative within the initial 2 months of treatment. The earliest sign of therapeutic activity detectable by CT or PET scan was observed on day + 12 of crizotinib. Seven patients achieved response to crizotinib (5 CR, 2 PR see table 1) for an ORR of 78% (95% C.I.: 44-93%), 1 patient achieved stable disease (SD). Disease status at the latest follow-up is as follows: 4 CR (months 8+-20+), 5 PRO (4 deaths due to progression). Three of the 5 patients who relapsed had previously undergone ABMT. One patient in CR1 stopped crizotinib and underwent a related alloBMT. The disease recurred on day + 76 after transplant. She restarted crizotinib and obtained CR2 in 14 days. Toxicities observed were mild and include grade I-II diarrhoea, rash and ocular flashes. **Conclusions**.Crizotinib exerted a potent antitumor activity in advanced ALK+ lymphoma and obtained durable response (4/9 patients or 44% 95% C.I.: 19-73%) even in this population of heavily pre-treated patients.

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MILATUZUMAB-AS A SINGLE AGENT IN REFRACTORY CLL- INTERIM RESULTS OF A PHASE I-II STUDY

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On behalf of the Israeli CLL study groupBackground. : Our previous work showed that the MHCII related protein-CD74, is over expressed from the very early stages of the disease. Activation of CD74 with its ligand MIF leads to survival signals in all stages[1]. Further more it also leads to increased VLA-4 expression with resultant homing to the bone marrow in advanced stages of the disease. We also showed that blocking CD74 with the humanized MoAb-milatuzumab leads to increased apoptosis and decreased migration to the bone marrow[2]. **Aims.** Based on our pre-clinical results the aim of our study was to assess the efficacy and toxicity of milatuzumab in advanced refractory CLL patients. **Methods.** Milatuzumab (120mg/m2) was given twice weekly for two months. After a four week wash out period, this was followed by escalating doses up to 600mg/m^2 for another 3 months. (See figure for details). Patients were then allowed to continue treatment as long as there were no significant adverse events or no further response.Results. So far, 6 patients with advanced refractory CLL, all heavily pretreated, were recruited. 3/6 showed significant improvement in their cytopenias, with no need for blood transfusions in 2 patients who were transfusion dependent prior to enrolment. 5/6 patients had significant improvement in B symptoms and functional level. None of the patients died or suffered significant treatment related toxicity during the study period. Patient samples were also studied and showed a decrease in bcl-2 , mcl-1 and VLA-4 mirroring what we have seen in the xenograft mouse model.Conclusions: Our results so far, combined with data from other studies using this agent in the USA suggest that it is safe and improves the quality of life and functional ability of frail elderly advanced stage CLL patients. It also suggests that it should be given continuously, as in most patients the effects were diminished after a short period of discontinuation of the agent. This was seen both in clinical and laboratory parameters. One patient received milatuzumab for over a year with no significant adverse effects.1. Binsky I, Haran M, Starlets D, et al. IL-8 secreted in a macrophage migration-inhibitory factor- and CD74-dependent manner regulates B cell chronic lymphocytic leukemia survival.

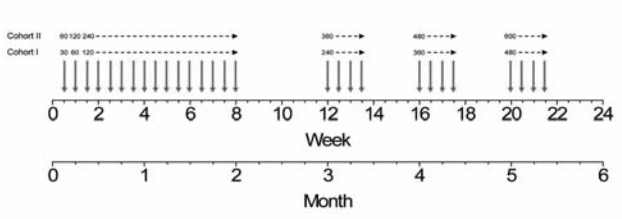

Figure 1. Milatuzumab treatment plan. The numbers to the left of top arrows are doses in mg per meter squared.

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1155

ECULIZUMAB THERAPY FOR PEDIATRIC PATIENTS WITH ATYPICAL HEMOLYTIC UREMIC SYNDROME: EFFICACY AND SAFETY OUTCOMES OF A RETROSPECTIVE STUDY

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Background. Atypical hemolytic uremic syndrome (aHUS) is a rare, genetic, and life-threatening disease driven by chronic, uncontrolled complement activation that results in multi-organ damage caused by thrombotic microangiopathy (TMA). Despite plasma exchange/infusion (PE/PI), patients with aHUS have a poor prognosis, with a 33%-40% rate of progression to end-stage renal disease or death with the first clinical aHUS manifestation. Within a year of diagnosis, 65% of aHUS patients will require dialysis, have permanent renal damage, or die. Chronic treatment with eculizumab, a terminal complement inhibitor, has been demonstrated to be safe and effective in 2 prospective clinical trials of adult/adolescent aHUS patients and was recently approved in the United States and Europe for treatment of pediatric and adult aHUS patients.**Aims.** Assess the efficacy and safety of eculizumab treatment for aHUS in pediatric patients within a medical-practice setting. **Methods.** Medical record data were retrospectively collected for 19 aHUS patients aged <18 years (<2y [n=5]; 2-11y [n=10]; 12-17y [n=4]) who received eculizumab between 2007 and 2009 outside of a controlled clinical trial setting.

Baseline Characteristics	Aged <12 Years (n=15)	Aged <18 Years (N=19)
Platelet count <150×10^9/L, n (%)	7 (47)	8 (42)
eGFR <60 mL/min/1.73 m^2, n (%)	9 (60)	13 (68)
Received dialysis within 4 weeks of 1st eculizumab dose, n (%)	6 (40)	8 (42)
Previous kidney transplantation, n (%)	3 (20)	6 (32)
Identified genetic complement mutation, n (%)	7 (47)	10 (53)
Efficacy Outcomes During Eculizumab Treatment		
Platelet normalization (>150×10^9/L)		
All patients, n (%)	14 (93)	17 (89)
Patients with abnormal platelet count at baseline, n/N (%)	6/7 (86)	7/8 (88)
eGFR improvement ≥15 mL/min/1.73 m^2, n (%)	8 (53)	9 (47)
TMA event-free status attained, n (%)	11 (73)	13 (68)
Median (range) TMA intervention rate (PE/PI or dialysis events/patient/day)		
Pretreatment period	0.45 (0-2.38)	0.31 (0-2.38)
Treatment period	0 (0-0.08)	0 (0-0.08)

eGFR=estimated glomerular filtration rate; PE=plasma exchange; PI=plasma infusion; TMA=thrombotic microangiopathy.

Results. Baseline data and eculizumab efficacy outcomes for the 19 patients aged <18 years and a subgroup of 15 patients aged <12 years are presented in the Table. Median duration of eculizumab therapy was 28 weeks (range=1-70wks). Eculizumab inhibited terminal complement activation in all patients for whom evaluation was requested by the treating physician. Eculizumab also reduced TMA as demonstrated by platelet count normalization in 17 (89%) of all 19 patients and in 7/8 patients (88%) with platelet count levels <150×10^9/L at baseline. Nine patients (47%) demonstrated kidney function improvement (estimated glomerular filtration rate increase ≥15 mL/min/1.73 m^2) and 4/8 patients (50%) eliminated the need for dialysis during eculizumab treatment. Thirteen patients (68%) achieved TMA event-free status (no PE/PI, no new dialysis, and no decrease in platelet count >25% from baseline for ≥12 weeks). TMA intervention rate (PE/PI or new dialysis events/patient/day) was reduced in all patients receiving PE/PI or dialysis prior to eculizumab treatment (median [range] TMA intervention rate=0.31 [0-2.38] pretreatment vs 0 [0-0.08] during eculizumab therapy). The most common adverse event during eculizumab treatment was pyrexia (n=9; 47%). Efficacy and safety outcomes were similar in the subgroup of patients aged <12 years, with kidney function improvement in 8 patients (53%), achievement of TMA event-free status in 11 patients (73%), and a reduction in TMA intervention rate (median [range] rate=0.45 [0-2.38] pretreatment vs 0 [0-0.08] with eculizumab) during eculizumab treatment. **Conclusions:** In this pediatric population (aged <18y), chronic eculizumab treatment inhibited terminal complement activation, reduced TMA, and improved kidney function, including elimination of dialysis in 50% of patients (4/8). Eculizumab treatment also reduced need for PE/PI in all patients who previously required PE/PI. The efficacy of eculizumab treatment was similar in both pediatric age groups and treatment was well tolerated. These findings are consistent with prospective clinical trial results of adult/adolescent aHUS patients, thereby supporting the role of eculizumab as a new standard of care for aHUS patients of any age.

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IMMUNOTHERAPY WITH THE TRIFUNCTIONAL ANTIBODY FBTA05 IN PEDIATRIC HIGH RISK PATIENTS WITH RECURRENT CD20 POSITIVE B CELL MALIGNANCIES - A CLINICAL FOLLOW UP

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Pediatric patients with B cell malignancies refractory to standard therapy have limited treatment options and display poor prognosis. Thus, novel treatment approaches are urgently required. Here we follow up ten children diseased with Non Hodgkin's Lymphoma (NHL) or acute leukemia being treated with FBTA05, a trifunctional anti-CD3 x anti-CD20 antibody in compassionate use. According to patient history, all children presented extensively pretreated and refractory to standard treatment (radiation, chemotherapy) including rituximab and allogeneic stem cell transplantation (five patients) before treatment with FBTA05.Within individual treatment schedules, two different treatment strategies were studied. Weekly applications of FBTA05 escalated up to 300µg after allogeneic transplantation were intended to provoke sustained remissions by FBTA05 induced cellular immune response. Daily applications, with escalated doses of FBTA05 up to maximal 1,000µg within one week were intended to harness the antibody´s cytotoxic potential to reduce tumour burden or to eradicate minimal residual disease before allogeneic transplantation.In two patients FBTA05 was combined with escalated doses of DLIs after allogeneic transplantation. In one child with NHL, FBTA05 and DLI were combined with lenalidomide within a second and third treatment cycle. All other children were treated stand alone with FBTA05 whereas in one case, subsequent to allogeneic transplantation additional weekly applications of FBTA05 were performed.Nine of the ten children displayed a clinical response: two stable diseases, two partial remissions and five complete responses (CR). Of note, in one of these patients a molecular CR was achieved after stand alone treatment with FBTA05. In follow up, overall survival (OS) is currently in the range of 25 up to 856 days and will be updated at time of presentation. Three out of these nine responders died, two children due to relapse or tumor progression (OS 59 and 129 days, respectively), one due to suspected pulmonary embolism (OS 829 days). The other patients still sustain in complete or partial remission.Within follow up analysis human anti-mouse antibodies (HAMAs) were detectable only in one case appearing four weeks after start of FBTA05 therapy. Interestingly, two additional applications of FBTA05 could be administered safely. Thereby HAMAs disappeared four months later. Graft-versus-host disease (grade III-IV) could be observed in two patients (in one case after DLI) and resolved by further immunosuppressive therapy.In general, FBTA05 applications were well tolerated by all children and adverse events were restricted to fever, chills and fatigue (one case). Combination of FBTA05 with lenalidomide was also well tolerated, but resulted in nausea and a transient aggravation of a preexistent leukocytopenia during the first cycle (10 and 15mg daily) which could be resolved by dose adjustment of lenalidomide to 5mg daily in the following cycles.Taken together, despite poor clinical prognosis of pediatric patients refractory to standard treatment, immunotherapy with FBTA05 resulted in a favorable clinical outcome including partial and complete remissions.

Thrombosis and vascular biology

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EGFP-EGF1 PROTEIN-CONJUGATED PEG-PLGA NANOPARTICLES ALLOW NF-κB DECOY DELIVERY INTO INJURED CEREBROVASCULAR ENDOTHELIUM TO PREVENT CEREBRAL THROMBOSIS

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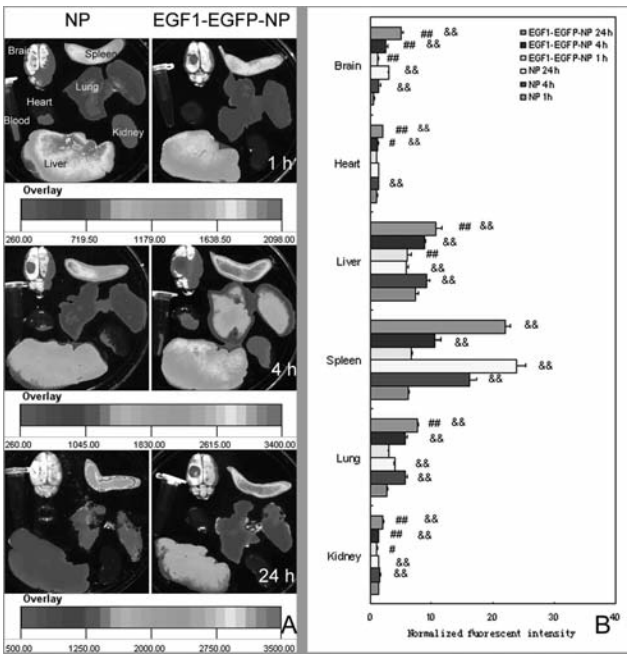
Background. Tissue factor (TF) interacts with either plasma coagulation factor FVII or FVIIa, and the resulting fibrin serves as a trigger for thrombosis formation. The transcription factor nuclear factor-kappa B decoy oligonucleotides (ODNs), which can inhibit TF expression in stimulated vascular endothelial cells. Recently, we reported the synthesis of EGFP-EGF1 protein-conjugated PEG-PLA nanoparticles and showed their accumulation in the thrombi upon caudal vein administration.



Methods. EGFP-EGF1 protein-conjugated PEG-PLGA nanoparticle (EGF1-EGFP-NP) was employed as a new vector, and ODNs was incorporated into EGF1-EGFP-NP, and the resulting EGF1-EGFP-NP-ODNs was evaluated as a vector for gene therapy of cortex infarction. **Results.** At 2 hours after transfection of TF expressed rat brain capillary endothelial cell (BCECs), EGF1-EGFP-NP-ODNs was more efficiently internalized and located in the cytoplasm compared with NP-ODNs. At 4 hours and 6 hours after transfection, respectively, released ODNs were present in the nuclei and obviously inhibited the TF expression induced by TNF-α. At 6 hours after i.v. administration of NIR dye Dir loaded NPs to photchemical Cerebral thrombosis rats, the mean fluorescence intensity of the EGF1-EGFP-NP group was more than 4 times that of the NP group at the illuminated regions and most EGF1-EGFP-NPs were distributed in the vessel wall, via absorptive-mediated transcytosis, accumulated in endothelium cells and lowered the TF expression. At 24 hours after i.v. administration of EGF1-EGFP-NP-ODNs, MR imaging of cortex infarcted volumes were predominantly dwindled. **Conclusions.** EGF1-EGFP-NP-ODNs is a promising candidate for noninvasive gene therapy of cerebral infarction.

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SINGLE-NUCLEOTIDE POLYMORPHISMS AND VENOUS THROMBOEMBOLISM: A DANISH FOLLOW-UP STUDY

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Background. Various single nucleotide polymorphisms (SNP) located in genes with a role in coagulation have been associated with a higher risk of venous thromboembolism (VTE). However, reproducibility of these associations has not been entirely successful and replication studies are warranted. **Aims.** We investigated the VTE risk related to SNPs in the genes for Glycoprotein VI on platelets (GP6 rs1613662 A>G), Antithrombin (SERPINC1 rs2227589 G>A), Factor XI (F11 rs2036914 T>C and F11 rs2289252 C>T), Fibrinogen (FGG rs2066865 C>T) and Factor XII (F12 rs1801020 C>T) in a Danish follow-up study. **Methods.** From 1993 to 1997, 57.053 persons between the age of 50 and 64 years and free from a prior diagnosis of cancer joined the Danish "Diet, Cancer and Health"cohort. The primary outcome of this study was a first-time verified VTE episode defined as deep venous thrombosis or pulmonary embolism, and participants with a diagnosis of VTE before enrolment were excluded. Blood samples were drawn from each participant at baseline and SNP genotypes were determined by allele specific real-time polymerase chain reactions (predesigned Taqman SNP genotyping assay from Applied Biosystems). A case-cohort design including all incident VTE cases and a randomly selected subcohort of 1841 participants from the whole cohort was applied. Cox proportional hazard model with age as time-axis was used for statistical analyses and adjustments were performed for sex, age and hormone therapy. Persons homozygous for the allele reported to be protective for VTE in previous studies were chosen for reference group.

Table. Associations between examined SNPs and venous thrombosis. *Hazard ratios adjusted for age, sex and hormone therapy (women only).

Gene and SNP	VTE cases	subcohort members	Hazard ratio* (95% CI)
GP6 (rs1613662)	n=600	n=1.742	
• G/G	2.0 %	2.8 %	1
• A/G	24.8 %	29.6 %	1.27 (0.65-2.47)
• A/A	73.2 %	67.7 %	1.60 (0.84-3.08)
SERPINC1 (rs2227589)	n=607	n=1.730	
• G/G	81.4 %	82.9 %	1
• G/A	17.3 %	16.1 %	1.11 (0.86-1.43)
• A/A	1.3 %	1.0 %	1.22 (0.52-2.86)
F11 (rs2036914)	n=594	N=1.733	
• T/T	18.7 %	24.1 %	1
• T/C	49.5 %	51.1 %	1.26 (0.98-1.62)
• C/C	31.8 %	24.8 %	1.65 (1.26-2.17)
F11 (rs2289252)	n=604	n=1.747	
• C/C	27.5 %	35.1 %	1
• C/T	52.6 %	50.1 %	1.34 (1.08-1.66)
• T/T	19.9 %	14.8 %	1.71 (1.29-2.27)
FGG (rs2066865)	n=597	n=1.726	
• C/C	50.3 %	55.7 %	1
• C/T	41.0 %	37.2 %	1.21 (0.99-1.48)
• T/T	8.7 %	7.1%	1.34 (0.94-1.92)
F12 (rs1801020)	n=606	n=1.731	
• T/T	5.1 %	5.4 %	1
• T/C	36.1 %	37.9 %	1.03 (0.66-1.60)
• C/C	58.7 %	56.7 %	1.16 (0.76-1.78)

Results. A total of 641 incident VTE cases were verified. Sufficient DNA was available for SNP genotyping in the majority of VTE cases and subcohort members. The table shows the number of cases and subcohort members included in each SNP analysis as well as the hazard ratios (HR) for VTE. Genotypes TT and CT of rs2289252 and genotype CC of rs2036914 (both located in the *F11* gene) were significantly associated with a higher VTE risk when using their corresponding low-risk genotypes as reference. For the remaining four investigated SNPs a trend towards a higher VTE risk corresponding to the number of risk allele copies was observed. Among the subcohort members the risk allele was also the common allele for rs2036914 (*F11*), rs1801020 (*F12*) and rs1613662 (*GP6*). **Conclusions.** Genotype CC of rs2036914 and genotype TT of rs2289252, both located in introns of the *F11* gene, displayed statistically significant associations with VTE (HRs≥1.65). The mechanism by which they affect blood coagulation could be through increased plasma levels of FXI, which has been related to higher VTE risk. Although we included more than 600 VTE cases, our study lacks power to detect weak associations especially if allele frequencies are low. This may explain that the measures of association were not statistically significant for the remaining four included SNPs.

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PROSPECTIVE TWELVE MONTH STUDY COMPARING RISK FACTORS FOR HOSPITAL ACQUIRED THROMBOSIS WITH COMMUNITY ACQUIRED THROMBOSIS

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Background. Venous Thromboembolism (VTE) is estimated to be responsible for approximately 25,000 deaths in England annually. Since 2010, The Department of Health (DoH) has mandated hospitals in England to undertake VTE risk assessment of their inpatients using a national tool. Appropriate thromboprophylaxis (TP) should then be administered. Additionally, Hospital acquired thrombotic (HAT) events should be investigated to detect whether a risk assessment occurred or the event could have been prevented, using a root cause analysis (RCA) process. HAT is defined as a VTE event occurring as an inpatient or within 90 days following discharge. Identifying HAT events in a timely manner has been difficult. Coding is inaccurate and is a discharge process. The radiology reporting and record system has provided an effective mechanism for identifying HAT events to enable RCA to occur. **Aims.** This study was developed to assess the risk factors from the national tool that are most highly associated with VTE locally. Also to detect whether there is a significant difference in risk profile between HAT events compared with community acquired VTE (CAT). **Methods.** Over twelve months from January 2011, the hospital computerised radiological information system (CRIS) was reviewed on a daily basis, to record VTE events. Specifically computed tomography pulmonary artery scans, ventilation/perfusion scans and doppler ultrasound scans of the lower and upper limbs were reviewed for positive results. Incidental VTE events were also collected from the CRIS system. The patient information system was cross-checked to detect which VTE events satisfied the criteria for HAT. The hospital records of all patients with VTE were then interrogated to assess the presence of DoH VTE risk factors.

RISK FACTORS	CAT DVT (255)	CAT PE (305)	HAT DVT (76)	HAT PE (165)	ALL VTE (801)
AGE	126 (49%)	183 (60%)	46 (61%)	122 (74%)	477 (60%)
CANCER	44 (17%)	73 (24%)	21 (28%)	59 (36%)	197 (25%)
SMC	74 (17%)	143 (47%)	22 (29%)	78 (47%)	317 (40%)
OBESITY	49 (19%)	21 (7%)	16 (21%)	20 (12%)	106 (13%)
PREVIOUS VTE	79 (31%)	57 (19%)	13 (17%)	15 (9%)	164 (20%)
FAMILY HISTORY VTE	37 (15%)	16 (5%)	0	2 (1%)	55 (7%)
PREGNANCY	1 (0.4%)	5 (2%)	1 (1%)	3 (2%)	10 (1%)
DEHYDRATION	0	0	8 (11%)	16 (10%)	24 (3%)
IVDU	26 (10%)	5 (2%)	3 (4%)	0	34 (4%)
NO RISK FACTORS	37 (15%)	55 (18%)	1 (1%)	2 (1%)	95 (12%)

Results. Table shows the breakdown of HAT and CAT events, comparing risk factors defined by the national tool. SMH is a significant medical co-morbidity, as defined in the DoH tool and IVDU is Intravenous drug use which was additionally included as a prevalent group in this patient cohort. In 2011 there were 801 VTE events (331 DVT & 470 PE) of which 241 (30%) met the HAT criteria (165 PE & 76 DVT). There was also a significantly increased association of HAT with PE compared to DVT (Chi-squared statistic 8.57, p value 0.0034)Age is the most common risk factor showing a greater relationship with HAT compared to CAT events. Cancer is significantly more associated with PE than DVT in both HAT & CAT groups. Medical co-morbidity appears to carry an equivalent risk for HAT and CAT but again a greater association with PE. Personal and family history of VTE, are more associated with CAT than HAT events and finally, not surprisingly, intravenous drug users are more associated with CAT and DVT. **Summary.** Risk factor profiles are different between CAT and HAT events, as well as PE compared to DVT. Of some concern, the ratio of PE to DVT is significantly greater in HAT events (2.7 v 1.2).16% of CAT events had no risk factors whereas only 1% of HAT events fell in this category.

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NOVEL ROLE OF OXYGEN SENSING MESENCHYMAL STEM/PROGENITOR CELLS AS INITIATORS OF NEO-VASCULOGENESIS *IN VIVO*

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Background. In response to hypoxic tissue damage endothelial colony-forming progenitor cells (ECFCs) are considered to perform new vessel formation and subsequently recruit mesenchymal stem/progenitor cells (MSPCs) to differentiate into stabilizing pericytes. Stem cell therapy to re-vascularize ischemic tissue has been a promising tool for various therapeutic targets including stroke, myocardial infarction and peripheral artery disease. Isolation and expansion of large quantities of stem/progenitor cells from different blood sources and bone marrow have set the first stone to meet this medical need. However, transplantation of endothelial without mesenchymal progenitors in a hypoxic environment failed to induce neo-vasculogenesis *in vivo*.**Aims.** We hypothesize that MSPCs have a primary role as oxygen sensors during vascular regeneration. Here we show for the first time that ECFCs in hypoxic conditions *in vivo* require the presence of MSPCs not only to stabilize but mainly to initiate neo-vasculogenesis. **Methods.** Adult human ECFCs were isolated from blood andMSPCs from bone marrow aspirates and expanded under humanized culture conditions. *In vitro* studies of progenitor cell phenotype, long-term proliferation, wound repair, migratory and vasculogenic functions were monitored at different oxygen conditions. ECFC and MSPC interaction *in vivo* were studied in immune-deficient NSG mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) after subcutaneous transplantation in various extracellular matrices (matrigel, collagen/fibronectin, human platelet lysate). To investigate the respective roles of MSPCs and ECFCs during vasculogenesis under hypoxia *in vivo,* chemical and genetic inhibitors of protein synthesis (cycloheximide) and HIF-1α (YC-1, shRNA) were employed. Immune histochemistry, immune fluorescence and an TUNEL assay were performed in time course after transplantation. **Results.** *In vitro* progenitor proliferation and function were reduced with declining oxygen levels. ECFCs stabilized hypoxia-inducible factor-1α (HIF-1α) only at 1% O_2, while MSPCs had already stabilized HIF-1α at 5% O_2 resembling venous oxygen conditions. In a immune-deficient mouse model, ECFCs transplanted into a hypoxic environment did not stabilize HIF-1α after 1 day and underwent apoptosis, while transplanted sole MSPCs showed strong nuclear HIF-1α stabilization. In co-transplanted ECFCs and MSPCs a subset of cells stabilized HIF-1α 1 day after transplantation and formed perfused human vessels within 7 days. Inhibition of HIF-1α stabilization in MSPCs significantly abrogated vessel formation *in vivo*, whereas HIF-1α inhibition in ECFCs could not circumvent vasculogenesis. Blocking the down-stream target of HIF-1α vascular endothelial growth factor (VEGF) resulted in an inhibition of neo-vasculogenesis.**Conclusions:** This data demonstrates that hypoxic ECFCs alone show reduced functionality *in vitro* and failed to form patent vessels *in vivo*. In contrast, MSPCs react to the low oxygen environment more sensitively than ECFCs and promote vessel formation at least in part by rescuing ECFCs from hypoxia-induced apoptosis. Surprisingly, this study shows that therapeutic vasculogenesis can occur independent of endothelial HIF stabilization. This data shows that MSPC and not ECFC

oxygen sensing is crucial to initiate vascular regeneration. This suggests a shift of focus from endothelial cells to perivascular cells as a therapeutic target in regenerative medicine and anti-angiogenic therapy.

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RELATIONSHIP BETWEEN SERIAL ULTRASOUND, D-DIMER AND FACTOR VIII AND DEVELOPMENT OF POST-THROMBOTIC SYNDROME FOLLOWING A FIRST DEEP VEIN THROMBOSIS

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Background. Post-thrombotic syndrome (PTS) is a common complication of deep vein thrombosis (DVT). Venous reflux and persistent obstruction are thought to contribute to the development of PTS. **Aims.** To evaluate the relationship between residual vein thrombosis (RVT), deep venous reflux, D-dimer and Factor VIII and development of PTS following a first DVT. **Methods.** 114 participants with confirmed DVT were recruited and followed for six months post completion of anticoagulation (or 12 months from diagnosis if anticoagulation continued). Ultrasound, D-dimer and Factor VIII were undertaken at six weeks following completion of anticoagulation (T1) and at end of follow up (T2). PTS was considered present in those with a score of ≥ 5 on Villalta scale at T2.Results. PTS was present in 40.4% of participants at end of follow-up. Those with PTS were significantly older (mean age 55.8 +/- 13.8 versus 42.4 +/-12.6 years, P<0.001) and had a higher body mass index (BMI: 30.7 compared 28.7, P=0.09). PTS was more common following proximal (47.4%) than distal DVT (33.3%, P=0.12). Median D-dimer in those with PTS was significantly higher at both time points (T1 480 IQR 300-750, T2 540 IQR 320 - 900) compared to those without (T1 310 IQR 240-490, P=0.008; T2 280 IQR 220-480, P<0.001). Similarly, Factor VIII measured at both time points was significantly higher in those with PTS (T1 176 IQR 145-238, T2 195 IQR 149 - 236) compared to those without (T1 150 IQR 130-200, P=0.032; T2 142 IQR 125-175, P<0.001). The presence of RVT was not significantly associated with PTS at either time point. PTS was more common in those with deep venous reflux at either time point (T1 73.7%, T2 70.6%) than those without (T1 32.3%, P=0.001; T2 33.0%, P=0.004). On univariable analysis of markers associated with PTS measured at T1, D-dimer >500ng/ml gave an odds ratio (OR) for PTS of 3.2 (95%CI 1.41- 7.35, P=0.005). Factor VIII >150u/dl was associated with an OR for PTS of 2.3 (95% CI 1.03- 5.02, P=0.04). The presence of deep venous reflux was associated with an OR of 5.88 (95% CI 1.94 - 17.84, P=0.002). Similar ORs were seen for these variables measured at T2. On multivariable analysis, after adjustment for age, BMI and location of DVT (proximal vs distal), only FVIII>150ng/ml and deep venous reflux at T2 were significantly associated with PTS. The OR for PTS associated with a high FVIII was 3.1 (95% CI 1.22 - 7.93, P=0.017) with the presence of deep venous reflux associated with an OR for PTS of 4.86 (95% CI 1.10 - 21.44, P=0.037). Summary and Conclusions. Factor VIII and deep venous reflux are independently associated with PTS at six months post end of anticoagulation but not earlier in the disease course. This supports the role of reflux in the pathophysiology of PTS. Increased FVIII activity suggests underlying activation of coagulation as a contributor to PTS.

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PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE IN PEDIATRIC PRECURSOR-B ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Minimal residual disease (MRD) has emerged as the most powerful tool for assessing response to chemotherapy in pediatric precursor B ALL. **Aims.** This study aimed to assess the presence of MRD in relation to other prognostic parameters such as age, gender, CSF status and molecular genetic abnormalities in pediatric precursor-B ALL. In addition, we aimed to clarify the impact of MRD status on disease progression. **Methods.** Bone marrow samples were obtained from 70 newly diagnosed pediatric ALL patients diagnosed in 2008-2011. Immunophenotypic characterization was performed at diagnosis. Cases were followed up for MRD at day 15 (d15) and 42 (d42). Leukemia associated phenotype(s) were identified using the following panels: CD58/CD10/CD34/CD19, CD38/CD10/CD34/CD19 and CD45/NG2/CD19/CD22 as well as any aberrant myeloid (CD13 or CD33) or T markers (CD7, CD2)/CD19/CD34/CD45. The cut off value used for MRD detection was 0. 01%. Patients were treated according to the Egyptian NCI treatment protocol modified from the total therapy study XV. Disease free survival (DFS) was evaluated at 28 months. **Results.** At D15 post-induction MRD was evaluated in 61 patients; 31 had MRD <0. 01, 20 had ≥0. 01<0. 1, 4 had MRD level ≥0. 1<1. 0%, 4 had MRD level > 1% and 2 patients did not achieve complete remission (CR). Associations between MRD at d15 post induction and other clinical and biological risk factors including age, gender, TLC, lymphadenopathy, hepatosplenomegly, cytogenetic molecular studies and DNA index were of no statistical significance. We reported statistically significant association between MRD positivity at d15 post induction and CSF infiltration (P value = 0. 03). The association between MRD, OS, and DFS at 28 months did not reveal statistical significance using different cutoff values of 0. 01 or 0. 1%. A trend of association with poor outcome was demonstrated with 0. 1% cutoff value though not statistically significant. At d42 post-induction MRD was evaluated in 56 patients; 32 had MRD level <0. 01, 19 had ≥0. 01<0. 1 and 4 patients had MRD level ≥0. 1<1. 0% and one patient had a level of > 1%. As with d15, we reported statistically significant association between MRD positivity at d42 post induction and CSF infiltration (P value = 0. 01) Also, significant association was demonstrated between MRD at d42 and molecular genetics; t(12;21) was significantly associated with negative MRD while t(9;22) was significantly associated with positive MRD (p=0. 045). Patients with negative MRD at d42 had significantly better DFS than those with positive MRD (p<0. 0001). No statistically significant association between d42 MRD and other prognostic parameters was encountered. **Summary and Conclusions.** Positive MRD is associated with some other bad prognostic parameters namely CSF infiltration and t(9;22). At day 42, MRD detection by flow cytometric assay using 0. 01% cutoff level is useful in predicting treatment outcome. The proven clinical value of MRD in the plethora of studies would raise a need for changing the current definition of CR, which is still based on the morphologic appearance of BM. Investigation of MRD identifies patients who will experience relapse in spite of a standard morphologic.

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PROGNOSTIC ROLE OF BLOOD COAGULATION FACTOR XIIIA EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Previously we have identified B-cell progenitor (BCP) lymphoblasts as a new expression site of coagulation factor XIII subunite A (FXIIIA). Detection of FXIIIA in BCP ALL blasts by flow cytometry (FC) can be used for more accurate definition on the leukemia-associated immunophenotype (LAIO) and quantitation of minimal residual disease (MRD). **Aims.** Here we have examined, for the first time, the possible impact of factor XIIIA expression in childhood BCP ALL. **Patients and Methods.** MRD detection was performed using four color FC analysis with a FACSCalibur flow cytometer. Sixty-one leukemic children with a BCP ALL phenotype were treated according to the antileukemic protocol BFM95/BFM ALL-IC 2002 and studied retrospectively. **Results.** Multiparametric data analysis of prognostic factors pointed on the possible role of FXIIIA expression to influence disease outcome. Unfavorable genetic conditions were significantly more frequent among FXIIIA-negative vs positive cases. Distribution of other conventional prognostic factors was similar between the two groups. Three-years overall survival of patients with FXIIIA positive disease (64%) was significantly higher than that of FXIIIA-negative patients (36%). **Conclusions.** Retrospective analysis of a limited number of patients indicated an important prognostic role of FXIIIA expression in childhood BCP ALL. Preliminary data suggest that FXIIIA expression may define a new subgroup of childhood ALL. Granted by TÁMOP-4. 2. 1/B-09/1/KONV-2010-0007 project, supported by the European Union, co-funded by the European-Social Fund.

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UTILITY OF A SINGLE MULTIPLEX REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) ASSAY FOR DETECTION OF THE COMMON CHIMERIC FUSION TRANSCRIPTS IN ACUTE LEUKEMIA PATIENTS FROM NORTH INDIA

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Background. Cytogenetic and molecular abnormalities identify prognostically relevant subgroups in B-cell acute lymphoblastic leukemia (ALL) and Acute Myeloid Leukemia (AML). Many western studies quote the incidence of these fusion transcripts to be 30-35% in B-ALL and around 40-45% in AML. However the data from Indian Sub-continent is limited. **Aims and Objectives.** The present pilot study was undertaken to detect the incidence of common chimeric fusion transcripts of t(12;21), t(9;22), t(1;19), t(4;11), t(8;21), t(15;17) and t(inv16) in adult and pediatric B-ALL and AML cases using a single Multiplex RT-PCR assay. **Methods.** This prospective study carried out over a period of one year included 95 B- ALL cases and 56 AML cases diagnosed on bone marrow (BM) examination and flowcytometric Immunophenotyping (FCM-IP) analysis. A single Multiplex RT-PCR assay was carried out using primers specific to the fusion transcripts. **Results.** Out of the 95 B-ALL cases enrolled in the study, 56 (59. 0%) were pediatric and 39 (41. 0%) adult cases. A total of 29/95 cases (30. 52%) showed positivity for the various fusion transcripts with 15/56 (26. 8%) pediatric and 14/39 (35. 9%) adult cases. Of the fifteen positive pediatric cases, 9/56 (16. 07%) were positive for TEL-AML1 transcript, 3/56 (5. 35%) for BCR-ABL transcript, 2/56 (3. 5%) for MLL-AF4 transcript and 1/56 (1. 79%) for E2A-PBX transcript. Out of the fourteen positive adult cases, 10/39 (25. 64%) were positive for BCR-ABL transcript, 2/39 (5. 12%) were positive for TEL-AML1 transcript and 1/39 (2. 56%) each for E2A-PBX transcript and MLL-AF4 transcript. Of the total 56 AML cases, 44 (78. 5%) were adult AML cases and 12 (21. 5%) pediatric cases. A total of 27/56 (48%) AML cases showed positivity for various fusion transcripts of which 18/44 (40%) were adult AML cases and 9/12 (75%) pediatric cases. Of the 18 positive adult cases, 8/44 (18%) each were positive for t (8;21) and t (15;17) and 2/44 (4. 5%) for t (inv 16). In the 9 positive pediatric cases, 5/12 (42%) were positive for t (8;21), 2/12 (16%) each for t (15;17) and t (inv 16). **Conclusions.** Our study results show that TEL-AML1 and AML1-ETO transcripts are the most common fusion transcripts seen in pediatric B-ALL & AML cases and BCR-ABL and AML1-ETO & PML-RARA in adult B-ALL & AML cases respectively. The incidence of the above common fusion transcripts in our pilot study is in accordance with that described in western studies. It is important to identify these transcripts as they provide useful prognostic information to the treating clinician.

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GERMLINE MUTATIONS IN MRE11/RAD50/NBN COMPLEX GENES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The *MRE11*, *RAD50*, and *NBN* genes encode proteins of the MRE11-RAD50-NBN (MRN) complex involved in cellular response to DNA damage and the maintenance of genome stability. In our previous study we showed that the germline I171V mutation in *NBN,* may be considered as a risk factor in the development of childhood acute lymphoblastic leukemia (ALL) and some specific haplotypes of that gene may be associated with childhood leukemia. This finding raises important questions about the role of mutations in others genes of the MRN complex in childhood ALL. **Aims.** The aim of the study was to answer the question whether *MRE11* and *RAD50* alternations have a potential role in childhood acute lymphoblastic leukemia. **Methods.** The aim was carried out by determining the frequency of constitutional mutations and polymorphisms in selected regions of *MRE11*, *RAD50*, and *NBN* in the group of 200 children diagnosed with ALL and treated at the Department of Pediatric Hematology, Oncology and Transplantology Poznan University of Medical Sciences in Poland. We have for the first time made simultaneous

analysis of *MRE11, RAD50* and *NBN* genes in childhood ALL. Anonymous blood samples collected on Guthrie cards were used as controls The analysis was focused on exon 5, 10, 14, 15, 19 of *MRE11*, exon 3, 4, 5 and 7 of RAD50 and 2, 5, 6, 7, 10, 13 of the *NBN* gene and was performed by specific amplification of region of interest by PCR and followed by multi-temperature single-strand conformation polymorphism (PCR-MSSCP) technique. Each sample showing shifts in MSSCP analyses was sequenced. **Results.** In cohort of the 200 children diagnosed with ALL we didn't identified any mutation neither in the *RAD50* nor in the *MRE11*. We identified 2 alternations in 3 out of 506 controls in the *RAD50* gene. The V127I in exon 4 of the *RAD50* gene, detected twice, was predicted to be tolerated using the SIFT and PolyPhen analysis. The V315L in exon 7 occurring only one among 506 controls was predict to be benign. In addition we were able to detect c. 551+19 G>A single nucleotide polymorphisms in the intronic sequence of exon 4 with a different frequency in ALL patients and controls. The frequency of the AA genotype was higher in leukemia patients, as compared to controls (p= 0,0032). In the pooled group of ALL patients (135 cases from previous study) we still observed the higher incidence of the I171V mutation in ALL group (5/200) than among controls (12/2400) (p< 0,0007). **Conclusions.** Although we observed variant allele of the intronic SNP in the *RAD50* gene as more frequent in childhood ALL in comparison to controls, no clear association of mutations in *RAD50* and *MRE11* gene has been seen. Interpretation of these results is limited by the rather low number of patients studied and needs further investigation. Nevertheless the obtained results confirmed the participation of the I171V germline mutation of *NBN* gene in childhood ALL. This research was supported by the grant from the Ministry of Sciences and Higher Education (NN407 201 737)

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AN ATYPICAL BCR-ABL TRANSCRIPT WITH AN INTRONIC SEQUENCE FROM THE BCR AND ABL GENES UNDETECTABLE BY EAC Q-RT-PCR IN A PATIENT ACUTE LYMPHOBLASTIC LEUKEMIA

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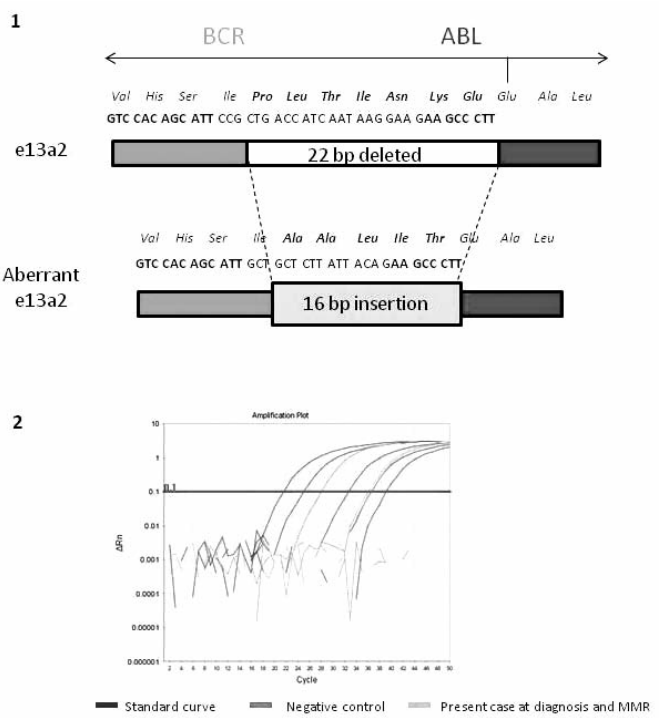
Background and Aims. The Philadelphia(Ph) chromosome arises from the reciprocal translocation t(9;22) (q34;q11) which produced the *BCR-ABL* chimeric gene. Very rare cases have been described with *BCR-ABL* breakpoints outside the well defined cluster regions. At diagnosis, the classical and unusual *BCR-ABL* variants can be successfully detected through sensitive techniques such as reverse transcriptase-polymerase chain reaction (RT-PCR) and/or fluorescence in situ hybridization (FISH). For minimal residual disease (MRD) monitoring, the quantitative RT-PCR (Q-RT-PCR) is required and is necessary for the management of patients with ALL or with chronic myeloid leukemia (CML) treated with tyrosine kinase inhibitors (TKI). To our knowledge, this is the first report of a PH+ALL with a rare e13a2 fusion transcript undetectable by EAC Q-RT-PCR but successfully diagnosed with a multiplex RT-PCR and by FISH analysis. **Materials and Methods.** According to the Multicenter GIMEMA studies for ALL, the diagnostic molecular analysis was performed in the reference laboratory of the Department of Hematology, University "Sapienza"(Rome). Total RNA was extracted using the Qiaquick gel extraction kit (Qiagen-Germany), and the *BCR/ABL* positivity was detected using the multiplex RT-PCR assay. FISH analysis was performed with LSI *BCR/ABL* dual color dual fusion translocation probe (Vysis; Abbott Molecular). The number of *BCR/ABL* copies was determined according to the EAC program methods. All Q-RT-PCR reactions were performed on a 7500 ABI platform (Applied Biosystems). Nucleotide sequencing was performed using an ABI PRISM 3130 genetic analyzer (Applied Biosystems, Foster City, CA). Sequence analysis was carried out using the basic local alignment search tool BLAST. **Results.** At diagnosis, the multiplex RT-PCR assay detected a *BCR/ABL* p210 positivity in the bone marrow sample with a PCR product sizing 397 bp which represents the b2a2 transcript; FISH confirmed the *BCR-ABL1* fusion. In contrast, by Q-RT-PCR the *BCR/ABL* junction could not be detected suggesting the presence of an aberrant transcript. Direct sequencing analysis of the RT-PCR product revealed that this patient harbored a novel form of aberrant e13a2 transcript located at 22 bases upstream of the 5'-terminal of the BCR gene and fused to the truncated ABL1gene in-frame with a 16-base insertion (Figure 1). This aberrant transcript lacked the fragment which contain the sequence recognized by the ENF 501primer, explaining why the Q-RT-PCR assay failed to detect the *BCR/ABL* positivity. For this reason, we designed a new forward primer located 41 nucleotides upstream from the breakpoint and this strategy allowed to successfully monitor the MRD levels of the patient (Figure 2) treated with Imatinib 800mg total dose plus steroids, which allowed to obtain a clinical remission with a 2 log Q-RT-PCR reduction from diagnosis. **Conclusions.** Our data demonstrate that qualitative, rather than quantitative, methods are mandatory for the detection of the *BCR/ABL* gene in Ph+ ALL, being able to detect also very rare cases with aberrant fusion transcripts. This is the first case among about 280 *BCR/ABL*+ ALL tested by EAC Q-RT-PCR that nonetheless indicates-as in CML- the possible pitfall of this strategy, at a time when the management of Ph+ ALL has dramatically changed following the advent of TKI.



Figures 1 and 2.

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A NATURAL PRODUCT, QUERCETIN ENHANCES THE CYTOTOXIC AND APOPTOTIC EFFECTS OF DEXAMETHASONE ON JURKAT CELLS

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Background. Quercetin, a flavonoid derived from red wine, onion, and tea, has important functions such as anti-inflammatory, anti-viral, immune system enhancer, anti-oxidant, and anti-cancer. Quercetin has cytotoxic effects on several cancer cells including leukemic, prostate, and breast cancers via several mechanisms such as cell cycle arrest at G_1 phase, induction of apoptosis, and inhibition of tyrosine kinase activity. In addition, quercetin also inhibits important oncogenes including c-myc and ras, while it upregulates p53. Dexamethasone, a glucocorticoid, is a drug having anti-inflammatory and immune suppressive effects. It is used in the treatment of several diseases such as rheumatoid arthritis, allergy, and asthma. Dexamethasone is also used for the treatment of hematological malignancies such as multiple myeloma and acute lymphoblastic leukemia. **Aims.** In this study, we aimed to determine possible synergistic apoptotic effects of quercetin and dexamethasone combination on Jurkat T-cell acute lymphoblastic leukemia cells. **Methods.** Cytotoxicity effects of quercetin and dexamethasone alone and their combinations on Jurkat cells were determined by MTT cell proliferation assay. Apoptotic effects of quercetin and dexamethasone alone or combination of both were assessed by examining the changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential, and the localization of phosphatidylserine on the plasma membrane. **Results.** MTT analyses showed that there were significant decreases in proliferation of Jurkat cells in response to quercetin and dexamethasone. IC50 values of quercetin and dexamethasone for 72 hours were calculated from cell proliferation plots and determined as 0. 7 and 9 μM, respectively. There were 1, 5, 6, 30 and 56% decreases in cell proliferation in response to 0. 1, 0. 5, 1, 5

and 10 μM dexamethasone, respectively, while combination of the same doses of dexamethasone with 0. 7 μM quercetin decreased proliferation of Jurkat cells 29, 36, 47, 63 and 72% respectively, as compared to untreated controls. In an attempt to confirm these data with apoptotic assays, the cells were treated with quercetin and dexamethasone alone or combination of both. The results revealed that there were significant apoptotic effects of combination of quercetin and dexamethasone compared to any agent alone or untreated control groups. There were 1. 0- and 1. 05- or 2. 88- and 3. 33-fold increases in loss of mitochondrial membrane potential in response to 0. 5- and 10 μM dexamethasone alone or the same doses of dexamethasone in combination with 0. 7 μM quercetin, respectively, while quercetin by itself induced loss of mitochondrial membrane potential 2. 12-fold. Furthermore, while apoptotic cell population treated with 0. 5 and 10 μM dexamethasone alone were increased 5 and 12%, combination of the same doses of dexamethasone with 0. 7 μM quercetin increased apoptotic cell population 85 and 228%, respectively, compared to untreated control group. Quercetin itself increased apoptotic cell population 27%. **Summary and Conclusions.** The results of this study demonstrated that there were synergistic cytotoxic and apoptotic effects of combination of dexamethasone and IC50 value of quercetin. Taking together these results showed that the use of quercetin in combination with dexamethasone may enhance the effectiveness of dexamethasone on Jurkat cells.

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GENE POLYMORPHISMS IN CRETAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Acute lymphoblastic leukemia (ALL) in children represents the most frequent form of malignancy. Several studies that have been conducted lately, have revealed an association between both the risk and the treatment outcome of ALL, with polymorphisms located in genes encoding for enzymes which are involved in various metabolic pathways. Such an example is the methylenetetrahydrofolate reductase (MTHFR), an enzyme which plays a role in the folic acid metabolism. Two common single nucleotide polymorphisms (SNPs) have been characterized in the *MTHFR* gene, at nucleotide positions 677 (C→T) and 1298 (A→C). Another case is the reduced folate carrier (RFC), an anion exchanger which transports both folate and methotraxate (MTX) into cells. The gene encoding RFC has been proved polymorphic at nucleotide position 80 (G→A). **Aims.** The presence of the *MTHFR* and *RFC* SNPs was examined in 35 children with ALL (14 females, 21 males-median age 6. 33 years) and in 48 healthy adults (control group), with Cretan origin. A comparison between the children's and the controls' genotype and allele frequency distributions was also performed. Moreover, the association between the *MTHFR* and *RFC* SNPs and the presence of MTX-related mucositis, hepatotoxicity and hematological toxicity, were also studied. **Methods.** The possible correlation between the SNPs in the genes examined and the susceptibility to ALL was examined using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) based approaches. The presence of hematological toxicity and hepatotoxicity was established from the average values of hemoglobin, platelets and white blood cells regarding the former and the average values of aspartate transaminase, alanine transaminase and gamma-glutamyltransferase regarding the latter. The laboratory values were each time calculated one week after the administration of IV MTX and the analysis was performed considering only higher grade toxicity. For statistical reasons and using the CTCAE(v4) from the NCI, children with normal laboratory values and no stomatitis, were labeled as having no presence of an adverse event, while children with toxicity grades 1-4, were labeled as having an adverse event. **Results.** The genotype and allele frequencies, regarding both SNPs in cases and controls, did not show any statistical differences. The results for the A1298C polymorphism, showed a statistically significant non-predisposing role of the A allele (A1298A+A1298C) regarding hepatotoxicity, as expressed by AST values (p=0. 039). The effect of the gene-gene interactions was also evaluated (*RFC/MTHFR* SNPs). The results revealed that the MTHFR A1298C / RFC G80A genotype, had a non-predisposing role regarding the susceptibility to ALL (p=0. 035), while the differences between the remaining haplotypes were not statistically significant. **Summary and Conclusions.** The results from our study of gene polymorphisms in children of Cretan origin with ALL may play a role in assessing the risk, both for the ALL susceptibility and the drug-induced toxicity, in our population. SNPs may be a tool in the ongoing effort of individualizing treatment, taking into account the genetic profile of each patient, the possible gene-gene interactions and the role of environmental factors and dietary habits.

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SIMULTANEOUS OCCURRENCE OF A ETV6-RUNX1 AND BCR-ABL (E1A2) TRANSCRIPTS IN A CHILD WITH A B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. According to the WHO classification (2008), the category of B-cell acute lymphoblastic leukemia (B-ALL) with recurrent genetic abnormalities comprises subtypes in which the presence of specific genetic abnormalities is associated with distinctive clinical or phenotypic properties, and clearly demonstrates that they are biologically distinct with important prognostic implications. The simultaneous co-existence of two of these aberrations in one and the patient are rare and raises questions concerning mechanisms of pathogenesis, exact sub-classification, risk group assignment and treatment policy. **Aims.** We present a rare case of simultaneous occurrence of a *ETV6-RUNX1* and *BCR-ABL* transcripts in a child with a B-cell acute lymphoblastic leukemia. **Case Report.** A 3-years-old boy presented with fever up to 38^{o}C, diarrhea, anemia and thrombocytopenia. Previous medical history included chicken pox and flu, respectively 4 and 2 months ago. On physical examination the patient was pale without hemorrhages The liver was enlarged, there was cervical micro-lymphadenomegaly, however no splenomegaly was detected. At the time of admission, laboratory tests revealed normal white blood cell counts of 5,9x10^{9}/l without blasts; hemoglobin level of 56 g/l and platelet counts of 40x10^{9}/l. On aspirate smears bone marrow was markedly hypercellular with a total infiltration with lymphoblasts. Flow cytometry of the bone marrow revealed a blast cells population accounting for 57% of all cells with a low FSC, low SSC, dim to negative expression of CD45 and B-II (pre-B) lymphoblastic phenotype: cytoplasmic CD79α(+), CD19(+), sCD22(+), CD10(+), CD34(+), CD20(-), sIgM(-), with aberrant dim CD13(+) and CD33(+) expression. Conventional cytogenetic analysis was unsuccessful. Molecular analysis using qualitative reverse transcription polymerase chain reaction according to the BIOMED-1 protocol revealed: negative results for *BCR-ABL* (p210); *MLL-AF4* and *E2A-PBX1* transcripts; and positive results *for BCR-ABL* (p190) and *ETV6-RUNX1* (long type) transcripts, which was confirmed in a second independently taken bone marrow specimen. Taking into account all of the above, a diagnosis of *BCR-ABL*-(p190)-positive and *ETV6-RUNX1*-positive B-cell precursor acute lymphoblastic leukemia was made and treatment was initiated according to the Protocol AIEOP-BFM-ALL 2000 for high-risk patients in combination with Imatinib 300 mg/m2 (since the 15-th day of induction therapy). A complete remission was achieved after the first course of treatment and 9 months after the diagnosis the child is alive with levels of minimal residual disease of 0. 036% (3,6x10-4) leukemia cells estimated by flow cytometry, 0. 035% (3,5x10^{-4}) *ETV6-RUNX1*/*ABL*, and 0. 023% (2. 3x10^{-4}) *BCR-ABL* (e1a2)/*ABL* transcripts evaluated by real-time quantitative RT-PCR. **Conclusions.** Up to our knowledge this is the first case reported with the simultaneous presence of *BCR-ABL*-(p190) and *ETV6-RUNX1* in a child with a B-ALL, that contributes to the heterogeneity of precursor lymphoblastic neoplasms. It does not demonstate any specific clinical, morphological or immunophenotypic features. The applied combined imatinib-chemotherapy approach seems to be effective allowing for MRD cleara

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IMMUNOPHENOTYPE, CYTOGENETICS AND MOLECULAR PROFILE OF TWO CASES OF ACUTE UNDIFFERENTIATED LEUKAEMIA

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Acute Undifferentiated Leukaemia (AUL) is a very rare type of leukaemia that shows no clear evidence of lymphoid or myeloid lineage specific antigen expression. It is classified within the Acute Leukaemias of Ambiguous Lineage in the WHO classification 2008. Little is known of the clinical features, genetics or prognosis associated with this type of leukaemia. The blast phenotype is defined as positive for CD45, HLA-DR, CD34 and/or CD38. TdT may be positive. Essentially the blasts do not express the T - or myeloid-lineage specific markers, cCD3 and MPO, and are negative for B-lineage specific markers such as cCD22 cCD79a or strong CD19. They lack specific features of other lineages such as megakaryocytic or antigens expressed by plasmacytoid dendritic cells. We diagnosed two

cases of Acute Undifferentiated Leukaemia with the above phenotype which were subsequently well characterised by cytogenetic and molecular studies. Peripheral blood and bone marrow was obtained from two elderly male patients, (83 and 84 years old). One of them had previous history of CLL and cutaneous DLBCL. The blasts were positive for CD45, HLA-DR, CD34, TdT, CD38 and CD123. Blasts from one of the patients were also positive for CD117 in the BM, but not in the blood. All T-, B- lymphoid and myeloid lineage markers were negative in both patients (cCD3, CD2, cCD22, cCD79a,CD19, MPO, CD13, CD33, glycophorin CD235a, CD41, and the plasmacytoid dendritic cell markers CD56 and CD4). Karyotype and FISH analyses showed 46XY +13, -21 in one of the patients; the karyotype of the second patient was normal. Molecular studies showed clonal rearrangement of the TCR beta chain and the Ig heavy chain gene DJH in both patients. One patient tested negative for NPMI/FLT3ITD. There is very little data in the literature on the genetic profile of these rare leukaemias. The molecular rearrangements suggest that they may arise from a very early lymphoid stem cell, although no myeloid-specific rearrangements have been tested. More cases need to be studied to attain a better understanding of the origin of AUL .

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LASER PROBING OF LYMPHOCYTES IN SOME BIOMEDICAL APPLICATIONS

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Conventional flow cytometry measures intensity of scattered light at two directions. Some advanced experimental devices, known as scanning (wide-angle) flow cytometers, measure angular dependences of intensity of scattered light ("cell fingerprints") over a wide interval of scattering angles. Such flow-cytometric light-scattering patterns give new opportunities for characterization of normal and pathological cells. Mononuclear cells of fresh peripheral blood were investigated in parallel for healthy donors, hepatitis B/C and acute lymphoblastic leukemia patients aged 26 to 48 y.o. The cells were isolated using Ficoll-Paque-TM PLUS separation solution. The purity of lymphocyte population isolated for analysis was approximately 94%. Computational modeling for single-lymphocyte light scattering was fulfilled. The findings showed that the scattering channels of flow cytometer provide opportunity of express assessment of cell optical pattern and allow to refer the cell to "normal", "viral" or "malignant" population without use monoclonal CD markers. Modeling data were validated by simultaneous flow-cytometry investigation. Both the experimental and modeling data demonstrated the utility of optical cell pattern ("cell fingerprint") in biomedical applications. Further investigations will show the potential value of light scattering approach in diagnosis and monitoring of acute blood malignancies.

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EXCELLENT OUTCOME AFTER T CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION WITH PRE-EMPTIVE DONOR LYMPHOCYTE INFUSION FOR PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA IN FIRST REMISSION

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Background. Approximately 25% of adult acute lymphoblastic leukemia (ALL) are Philadelphia chromosome positive (Ph^+) and express the *BCR-ABL protein* resulting from the t(9;22) translocation. Despite polychemotherapy Ph^+ALL is associated with a poor overall prognosis, with a median survival of 8 months. Although tyrosine-kinase-inhibitors (TKI) can provide additional disease control, most patients eventually relapse. Allogeneic hematopoietic stem cell transplantation (HSCT) in ALL is being performed to provide a graft-versus-leukemia (GVL-) effect, but at the cost of acute and chronic graft-versus-host-disease GVHD). T-cell-depletion (TCD) can prevent GVHD, but is associated with higher relapse rates. Pre-emptive donor T-lymphocyte infusion (DLI) may reintroduce GVL-reactivity with limited GVHD. Since the alloreactive GVL effect appears to be independent of the molecular risk profile, we hypothesized that also in Ph^+ALL TCD-HSCT followed by pre-emptive DLI and additional disease control with temporary TKI treatment may be beneficial. **Aims.** In this study, we compared the results of TCD-HSCT followed by pre-emptive DLI in patients with Ph^+ and Ph^- ALL in first complete remission (CR1). **Methods.** Twenty-two patients underwent myelo-ablative TCD-HSCT for ALL in first complete remission at LUMC between September 2003 and June 2010. Patient with GVHD needing systemic immune-suppression (IS) did not receive pre-emptive DLI. Patients without GVHD were scheduled to receive DLI between 3 and 6 months after alloSCT. Dosing of DLI depended on time after HSCT. Patients with a related donor (RD) were scheduled to receive up to 3. $0x10^6$ $CD3^+$cells/kg and patients with an unrelated donor 1. $5x10^6$ $CD3^+$cells/kg. **Results.** Eight patients had Ph^+ALL (36%). Seven Ph^+ALL patients received additional imatinib before HSCT of whom 3 also received imatinib after HSCT. Another 8 patients were diagnosed with (Ph^-) B-precursor ALL and 6 with T-ALL. Median time from diagnosis to HSCT was 175 days for Ph^+ALL *vs* 190 days for Ph^-ALL patients (p=0. 393). Five Ph^+ALL patients and 8 Ph^-ALL had a RD, respectively (p=0. 810). Median follow-up after HSCT is 63 months. Three of 8 Ph^+ALL patients developed chronic GVHD. The remaining 5 patients received DLI (median time-to-DLI 180 days). Hereafter, one did not convert to full-donor chimerism (FD) and developed a molecular relapse, which was successfully treated with DLI, interferon-alpha and nilotinib. None died during follow-up. None of 14 Ph^-ALL patients developed chronic GVHD. Three patients relapsed and 2 died of NRM within 6 months, before DLI was installed. Two did not receive DLI due to physician's choice. The remaining 7 received DLI (median time-to-DLI 189 days). One patient died 4 days later due to NRM. Another did not convert to FD and relapsed. One-year OS for Ph^-ALL was 50% (95% CI: 23-77%). Overall, OS of Ph^+ALL was superior to Ph^-ALL (log-rank p=0. 022, see Figure 1). Only 2 patients (9%) developed grade 3-4 acute GVHD after HSCT. After DLI, none developed ≥grade 2 acute GVHD, but one patient developed extensive chronic GVHD requiring IS. **Conclusions.** This study with long follow-up shows that myelo-ablative TCD-HSCT with pre-emptive DLI in CR1 abolishes the increased mortality associated with Ph^+ALL compared to Ph^-ALL in the era of BCR-ABL inhibitors.

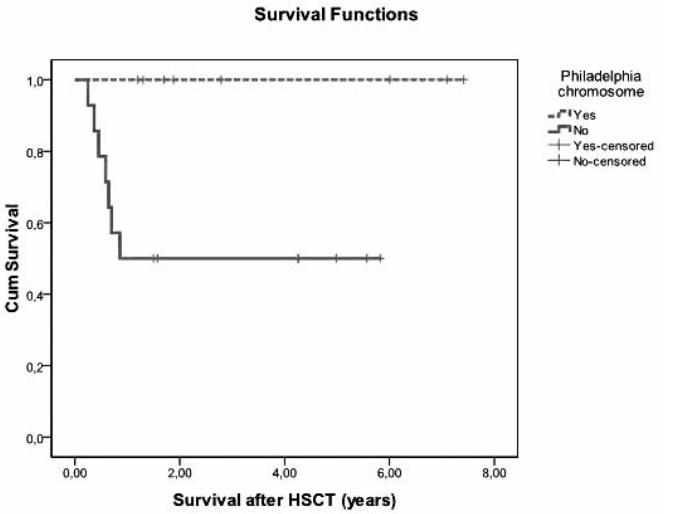

Figure 1. Overall survival Ph-positive ALL versus Ph-negative ALL.

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HIGH FREQUENCY OF BCR-ABL AND MLL-AF4 FUSION ONCOGENES IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS IS CORRELATED WITH DISEASE BIOLOGY AND POOR SURVIVAL

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Background. ALL is a complex genetic disease involving many prognostically important fusion oncogenes (FGs) [1], frequency of which can vary in different ethnic groups[2, 3] thus having implication in differential diagnosis, prognosis and

treatment. **Aims.** The objective of this study was to find out the frequencies of common fusion oncogenes in ALL and their correlation with disease biology and treatment outcome. **Methods.** We studied FGs in 101 pediatric ALL patients managed at Children Hospital, Jinnah Hospital and INMOL Hospital in Lahore, Pakistan. We used interphase-FISH and RT-PCR[1] at diagnosis (Day 0), and RT-PCR for follow up on Day 15 and Day 29, and studied the association of FGs with clinical features and treatment outcome. **Results.** Five most common FGs i. e. BCRABL (t; 9:22), TCF3-PBX1 (t; 1:19), ETV6-RUNX1 (t; 12:21), MLL-AF4 (t; 4:11) and SIL-TAL1 (Del 1p32) were found in 89/101 (88. 1%) patients. Survival of patients with >50,000 platelets count was significantly higher than patients with <50,000 platelets count (P=0. 013). Frequency of BCR-ABL gene was 44. 5% (45/101). Patients with BCR-ABL had significantly higher TLC count as compared to other patients and significantly lower survival (43. 7 ± 4. 24 weeks, p=0. 004) as compared to patients with other fusion genes, except MLL-AF4 (Figure 1). Overall survival for the whole group was 52. 2±3. 75 weeks. Survival of the patients harbouring ETV6-RUNX1fusion gene was better (65. 2± 9. 9 weeks, p=0. 009). The overall poor survival may be attributed to high frequency of poor prognostic fusion oncogenes (71%) and suboptimal supportive care.

Case Processing Summary

Oncogene	Total Number	Number of Events	Censored	
			N	Percent
BCR-ABL	45	30	15	33.3%
ETV6-RUN X 1	18	13	5	27.8%
MLL-AF 4	18	11	7	38.9%
SIL-TALL 1	6	5	1	16.7%
TCF3-PBX 1	2	1	1	50.0%
Overall	89	60	29	32.6%

Means and Medians for Survival Time according to oncogenes

Gene	Median			
			95% Confidence Interval	
	Estimate	Standard Error	Lower Bound	Upper Bound
BCR-ABL	40.000	2.683	34.741	45.259
ETV6-RUN X 1	56.000	5.392	45.431	66.569
MLL-AF 4	36.000	4.255	27.661	44.339
SIL-TALL 1	96.000	21.909	53.059	138.941
TCF3-PBX 1	72.000	.	.	.
Overall	48.000	5.110	37.985	58.015

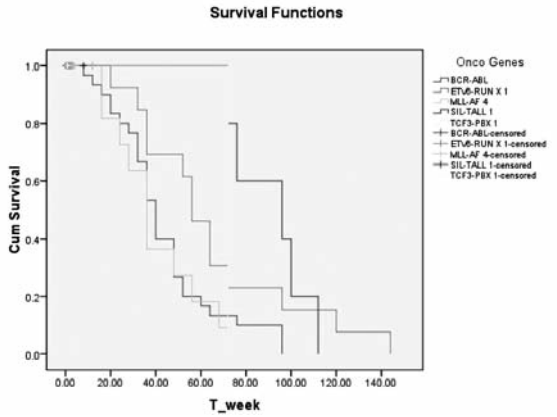

Figure 1. Frequency of different fusion oncogenes in pediatric acute lymphoblastic leukemia and their correlation with survival (Survival of patients with "ETV6-RUNX 1" is significantly higher than patients with other oncogenes. (p-value 0.009). BCR-ABL and MLL-AF4 genes have the lowest survival).

Summary and Conclusions. This is the first study from Pakistan correlating molecular genetic markers and disease biology to treatment response. It shows the highest frequency of BCR-ABL in pediatric ALL reported so far. Some authors have reported BCR-ABL frequency higher than Western populations[2, 4, and 5] while others reported 45% frequency of ETV6-RUNX1[6]. These and our data reflect strong interplay of genetic and environmental factors in the biology of pediatric ALL and its correlation with response to treatment [2, 3]. Our data also indicate an immediate need for large clinical trials to study the efficacy of tyrosine kinase inhibitors in paediatric ALL treatment in this ethnic group. This study will help unravel the role of BCR-ABL in leukemogenesis, and to find population-specific biomarkers and drug targets.

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THYROID DYSFUNCTION IN LONG-TERM SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOMA: EXPERIENCE OF A SINGLE ITALIAN PEDIATRIC INSTITUTION

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Background. The risk of thyroid dysfunction increases in long-term childhood survivors treated with radiotherapy (RT), due to disruption of the hypothalamic-pituitary axis during craniospinal RT or direct injury to the thyroid gland. There are few reports in the literature on the contribution of chemotherapy alone to the development of central hypothyroidism in childhood cancer survivors. **Aims.** We retrospectively evaluated the incidence of thyroid dysfunction in 397 long term survivors affected by lymphomas (97 patients) or acute lymphoblastic leukemia (ALL; 300 patients). Three hundred and six children were treated with chemotherapy alone and 91 received also cranial/craniospinal RT (43 ALL) or local RT (48 HD). All patients were treated in according with the protocols of the Italian Association of Pediatric Hemato-Oncology (AIEOP). **Methods.** From June 1986 to January 2012, 48 (30 M/18 F) Hodgkin lymphoma (HD), 49 (34 M/15 F) non-Hodgkin lymphoma (NHL) and three hundred (149 M/151 F) ALL long-term survivors were followed in a single pediatric Hemato-Oncology Institution. All patients annually underwent thyroid echography and blood measurement of free T4 and T3; TSH, anti-thyroperoxidase, and anti-thyroglobulin antibodies. All thyroid nodules exceeding 1,5 centimetres were aspirated and cytologically analyzed. **Results.** In our cohort of 397 long-term survivors, the average follow-up duration from completion of therapy to last follow-up was 7. 1 years (range, 1-26 years). Three out of 48 HD long-survivors (6,2%) developed thyreopathy (1 hypothyroidism, 1 nodules and hypothyroidism, 1 secondary neoplasm); all patients were treated with RT (respectively, 2 local RT followed by involved field after auto BMT and 1 total field). One out of 49 NHL (2,0%) survivors experienced thyroid nodules with normality of thyroid function; all patients were treated with chemotherapy alone. No ALL patients treated with cranial RT experienced thyreopathy; ten (2 males and 8 females) out of 257 ALL patients (3,9%), treated with chemotherapy alone, reported thyroid dysfunction. Six developed one or more thyroid nodules without lost of gland function (only one with thyroperoxidase and thyroglobulin antibodies). The fine needle aspirate cytology resulted negative for neoplasm. Subclinical hypothyroidism was observed in two patients. One patient developed autoimmune hypothyroidism and started thyroid replacement therapy while another reported autoimmune hyperthyroidism treated with methimazole. In two patient, familiar anamnesis was positive for thyreopathy. The mean time to thyroid dysfunction after stop-therapy was 3. 4 years (range, 1-12. 9 years); the mean age at the event was 13. 2 years (range, 6. 9-22 years). Table 1 resumes the clinical characteristics of survivors with thyreopathy.

Table 1. Clinical characteristics of patients.

Pts	Sex	Dx	Age at dx	Age at ST	RT	Thyreopathy	Thyroid andibodies	Thyroid function	TTEFST	Age at event	Need for therapy	Familiarity
1	M	HL	6 yrs 9 mos	8 yrs 1 mos	Yes	Thyroid cancer*	Yes	Altered[#]	5 yrs	13 yrs 2 mos	Yes	Adopted
2	F	HL	13 yrs 7 mos[§]	17 yrs 3 mos	Yes	Thyroid nodules	No	Altered	3 yrs[§]	17 yrs 7 mos[§]	Yes	No
3	M	HL	14 yrs	15 yrs 2 mos	Yes	Increase of TSH	No	Altered	2 yrs	17 yrs	Yes	No
4	M	NHL	8 yrs 1 mos	10 yrs 1 mos	No	Thyroid nodule	No	Normal	3 yrs	13 yrs 1 mos	No	No
5	F	ALL	8 yrs	10 yrs	No	Thyroid nodules	Yes	Normal	5 yrs	15 yrs	No	No
6	F	ALL	6 yrs 5mos	8 yrs 5mos	No	Hypothyroidism	Yes	Altered	1 yrs	9 yrs 5 mos	Yes	No
7	F	ALL	2 yrs 5 mos	4 yrs 5 mos	No	Thyroid nodule	No	Normal	3 yrs	7 yrs 5 mos	No	Yes
8	F	ALL	9 yrs 5 mos	11 yrs 5 mos	No	Thyroid nodule	No	Normal	4 yrs	15 yrs 5 mos	No	Adopted
9	F	ALL	2 yrs 9 mos	4 yrs 9 mos	No	Thyroid nodules	No	Normal	12 yrs	16 yrs 9 mos	No	No
10	F	ALL	4 yrs 9 mos	6 yrs 9 mos	No	Increase of TSH	No	Normal	1 yrs	7 yrs 9 mos	No	No
11	M	ALL	4 yrs 10 mos	6 yrs 10 mos	No	Thyroid nodules	No	Normal	4 yrs	10 yrs 10 mos	No	No
12	M	ALL	2 yrs 10 mos	4 yrs 10 mos	No	Increase of TSH	No	Normal	2 yrs	6 yrs 10 mos	No	No
13	F	ALL	10 yrs 11 mos	12 yrs 11 mos	No	Hyperthyroidism	Yes	Altered	9 yrs	22 yrs (or so)	Yes	No
14	F	ALL	7 yrs 7 mos	9 yrs 8 mos	No	Thyroid nodule	No	Normal	8 yrs	17 yrs 8 mos	No	Yes

Pts: patients
Dx: diagnosis
ST: stop therapy
RT: radiotherapy
TTEFST: time to event from stop therapy
*: papillary thyroid cancer
#: hypothyroidism after thyroidectomy
§: 4 months from stop therapy after second relapse

Conclusions. In our experience, the incidence of thyroid dysfunction in long-term pediatric survivals treated with RT was higher than in patients treated with chemotherapy alone. The more frequent thyroid abnormalities observed were thyroid nodules without gland failure. The short series of radiotherapy-treated survivors do not allow a correct evaluation of thyroid cancer incidence in these cohort of patients. We believe that childhood long-term survivors require lifelong surveillance after completion of therapy, for an early recognition and prompt treatment of late thyreopathy.

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BENDAMUSTINE PHARMACOKINETICS (PK) IN PEDIATRIC PATIENTS WITH RELAPSED/REFRACTORY ACUTE LEUKEMIA

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Background. The PK profile of bendamustine in adult patients is well characterized. Bendamustine is an investigational drug in pediatric acute leukemia. **Aims.** The objective of this analysis was to describe the pediatric PK profile of bendamustine relative to the adult PK profile and correlate the systemic exposure of bendamustine to efficacy and safety parameters in pediatric patients. **Methods.** Samples were obtained after a single dose from patients aged 1-19 years with relapsed/refractory acute leukemia who were enrolled in an open-label, nonrandomized study of bendamustine (90-120 mg/m^2, infused over 60 minutes). Caregivers/patients provided informed consent. Samples were obtained prior to bendamustine infusion and at preselected time points through 24 hours after start of infusion on day 1. Because cytochrome P450 1A2 (CYP1A2) is the principal enzyme that forms the two active metabolites (γ-hydroxy-bendamustine [M3] and *N*-desmethyl-bendamustine [M4]) in the liver, the effect of concomitant administration of medications known to be CYP1A2 inhibitors/inducers on bendamustine exposure was assessed. Other known parameters influencing systemic exposure (eg, kidney and liver function) were also assessed. Population PK modeling was performed using plasma concentration data from the pediatric patients. PK data from adults were used for comparison.

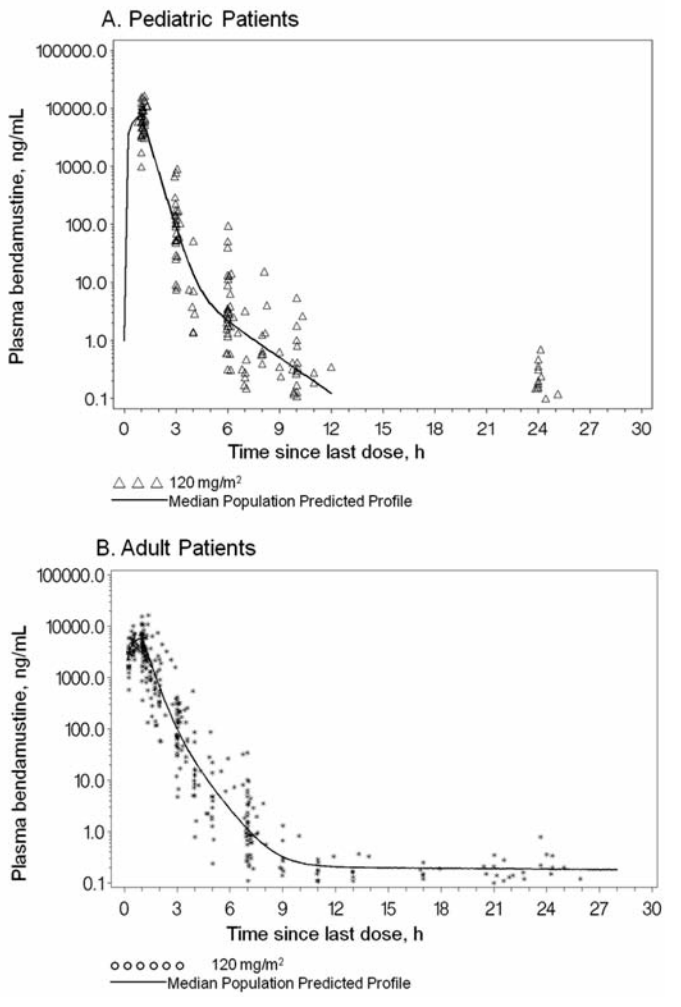

Figure 1. Bendamustine Systemic Exposur.

Results. Systemic exposure of pediatric patients to bendamustine was similar to that obtained previously in adult patients (see Figure 1, pediatric patients in panel A and adult patients in panel B). After a dose of 120 mg/m^2, mean maximum concentration (C_{max}) was 6806 ng/mL and mean area under the concentration-time curve from time 0 to 24 hours (AUC_{0-24})was 8240 ng-hr/mL in pediatric patients, compared with a mean C_{max} of 5746 ng/mL and AUC_{0-24} of 7121 ng-hr/mL in adults. The similarity in exposure despite the large range of body surface area across the pediatric and adult populations confirms the appropriateness of the body surface area-based dosing scheme. In pediatric patients, age, race, sex, or disease state had no statistically significant effect on systemic exposure to bendamustine. No changes in systemic exposure to bendamustine in the presence of a CYP1A2 inhibitor were observed. Differences in PK were not observed in pediatric patients with mild renal impairment, compared with patients with normal renal function; however, because only 3 patients had mild renal dysfunction, study data did not allow for a conclusive assessment of the effect of mild renal dysfunction on systemic exposure measures of bendamustine. Exposure in 2 pediatric patients with moderate hepatic dysfunction appeared to be higher. No clear exposure-response relationship was observed. Of fatigue, nausea, vomiting, and infection, infection was the only adverse event for which the probability of occurrence increased with increase in exposure to bendamustine. **Summary and Conclusions.** The PK profile of bendamustine in pediatric patients was similar to the known PK profile in adults, demonstrating that exposures reflective of the therapeutic range in adults were attained following administration of 120 mg/m^2 to pediatric patients. Support - Teva Pharmaceutical Industries Ltd.

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TIME COURSE OF ADRENOCORTICAL RECOVERY AFTER GLUCOCORTICOID THERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA:COMPARISON BETWEEN TWO DIFFERENT PROTOCOLS

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Background. Glucocorticoids are essential in protocols of therapy of acute lymphoblastic leukemia (ALL). Among the adverse effects of prolonged glucocorticoid therapy, adrenal insufficiency secondary to hypothalamic -pituitary - adrenal axis (HPA)suppression is a cause of concern. **Objectives of the study.** To assess the incidence, severity,morbid effects, and risk factors for HPA suppression in children with ALL, and the time course of recovery. **Study Design.** The study is a prospective study including 40 newly diagnosed patients with ALL diagnosed and followed in the Pediatric Hematology/Oncology Unit, Ain Shams University,Egypt. Prior to 2006, ALL patients were on modified BFM-1990 protocol; since 2006 treatment was changed to CCG based protocols . Accordingly, patients were classified according to the type of corticosteroid into: dexamethazone (DXM) group: 20 ALL patients on (CCG-1991) protocol, received DXM in induction I and II;and the prednisone (PDN) group: 20 ALL patients,received prednisone in induction I and II phases of modified BFM-1990 protocol. Patients were followed for glucocorticoid related complications especially life threatening adrenal decline, the steroid withdrawal syndrome, septic episodes, severe neutropenia, and associated drug therapy mainly fluconazole . The basal morning and the stimulated values of s. ACTH, cortisol, DHEAS were assessed, and the Low Dose Adrenocorticotropic hormone LD-ACTH (1mcg) stimulation test was done in all 40 patients at diagnosis, and, on weeks 0, 2 & 4 after steroid tapering. It was repeated every 2 weeks till adrenal recovery with additional testing during febrile and/or stress episodes. **Results.** Asymptomatic adrenal insufficiency was detected in 27. 5% of patients before treatment. Symptoms and signs of steroid withdrawal syndrome developed in 50% of PDN group and 75% of DEXA group after induction phases I and II; they occurred on days 1-3 and on days 4-9 following DXM and PDN tapering respectively . The HPA axis recovery assessed by serum cortisol and ACTH was earlier in PDN compared to DEX group($p<0.05$); 65% and 75% of patients on PDN recovered on week 2, while 45% and 50% of DXM group recovered in week 4 following tapering of steroids over 9 days in the induction phases I and II, respectively. Adrenal recovery was predicted 2 weeks earlier by normalized s. DHEAS levels. Gender did not affect the timing of recovery. Children below 5 years of age showed significant earlier recovery in induction phases I and II in PDN group compared to older age ($p=0.004$); there was no age effect in DEX group. Patients who had adrenal suppression prior to therapy had significantly late recovery ($p<0.001$). Increasing number of intercurrent infections were associated with late recovery in induction II in both groups. Fluconazole therapy ≥10mg/kg/day was significantly associated with longer duration to recovery in both groups in induction phases I and II. **Conclusions.** Prolonged adrenal suppression and related clinical morbidities is an inevitable consequence of high dose corticosteroid therapy in ALL children. Laboratory monitoring of cortisol levels, and, steroid coverage during periods of stress is recommended. A more gradual tapering of steroid therapy might be suggested.

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OUTCOME OF REINDUCTION TREATMENT USING MODIFIED DOSE OF IDARUBICIN FOR CHILDHOOD RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Treatment of marrow-relapsed childhood acute lymphoblastic leukemia (ALL) remains a great challenge. During reinduction treatment, induction failure and treatment-related toxicity are two major causes of treatment failure. Optimal intensity regimen is required to achieve the best remission induction rate and to minimize treatment-related mortality (TRM). **Aims.** This multi-center trial (CALL-0603) from Korea was conducted to develop an effective reinduction regimen with minimal toxicity by modifying the dose of idarubicin according to bone marrow suppression. **Methods.** Between 2006 and 2008, CALL-0603 trial accrued patients aged from 1 to 21 years with first isolated or combined marrow-relapsed ALL prospectively. Reinduction protocol consisted of oral prednisolone 60 mg/m^2/day for 28 days, weekly intravenous vincristine 1. 5 mg/m^2, intramuscular L-asparaginase 6000 IU/m^2 thrice weekly with a total of 9 doses and weekly intravenous idarubicin 10mg/m^2 on day 0, 7, 14, 21. If complete blood count on day 14 or day 21 showed absolute neutrophil count < 500/mm^3 or platelet count < 50,000/mm^3, administration of idarubicin was withdrawn. **Results.** A total of 38 patients were enrolled in the trial. The median ages at initial diagnosis and relapse were 8. 5 and 12. 0 years, respectively. Median duration from diagnosis to relapse was 23 months. Nineteen patients relapsed during initial treatment, 5 patients relapsed less than 6 months after the cessation of initial treatment, and 14 others relapsed more than 6 months. The second remission rate after 4 weeks of reinduction therapy was 74%, or 28 of 38 patients. Three patients achieved remission with extended or repeated reinduction treatment, resulting in the final remission rate of 82%. Median dose of idarubicin administered was 30 mg/m^2. Remission rate by idarubicin dose less or more than 20 mg/m^2 were not significantly different (69% vs. 77%, *P*=0. 713). Documented or suspected infection developed in 28 (74%), and 2 of them died of septic shock in refractory state during the reinduction attempt, resulting in the TRM rate of 5%. Length of initial complete remission less than 24 months was a marginally significant predictors of failure to enter second remission (*P*=0. 067). Twenty one patients received allogeneic hematopoietic stem cell transplantation (HSCT). Ten of them died of relapse after HSCT, and 5 of them died of transplant-related causes. The 3-year overall survival rate was 17. 5%. Duration of first remission and achievement of second remission after 4 weeks of reinduction treatment were significant predictors of long-term survival, while the total doses of idarubicin, and allogeneic hematopoietic stem cell transplantation were not. **Conclusions.** Four-drug reinduction treatment with a modified dose of idarubicin according to bone marrow suppression resulted in a favorable second remission rate in the treatment of relapsed ALL, which was due to lower TRM. However, despite a favorable remission rate, overall survival rate was unsatisfactory. More effective post-remission therapy should be investigated.

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THE INFLUENCE OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) POLYMORPHISMS ON THE SEVERITY OF MEDIUM DOSE-METHOTREXATE INFUSION RELATED TOXICITY AND PROGNOSIS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The over-all survival of acute lymphoblastic leukemia (ALL) of childhood reached to approximately 80% of the patients. Recent approaches focused to cure patient with minimal treatment associated morbidity and mortality. MTHFR, a fundamental enzyme that regulates folate metabolism, recently serves as a binoculer of a telescope to the pharmacogenetic world. Methotrexate (MTX), which interrupts folate metabolism, is widely used in the treatment of a variety of diseases including ALL, but causes some undesirable toxic effects especially on mucosal epithelia. **Aims.** We aimed to investigate the association of the MTHFR gene (C677T) polymorphisms with prognosis and toxicities after medium dose MTX (1000 mg/m^2/36h) infusion. **Methods.** We investigated the relationship between MTX related neutropenia and mucositis and MTHFR polymorphism. Toxicities were assessed using the Common Terminology Criteria for Adverse Events (CTCAE), Version 3. 0, published by the National Cancer Institute. The registration included nadir laboratory values [white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin level (Hb), platelet count (PLT), maximum plasma values of alanine aminotransferase (ALT)], occurrence of fever (hospitalization with temperature>38ºC), and delay in the chemotherapy regimen. **Results.** Of 85 enrolled children (54 boys and 31 girls; mean age 6. 3±3. 6, range 1-16 year) with newly diagnosed ALL treated to the Turkish national BFM ALL protocol at Erciyes University, Children's Hospital, Kayseri, Turkey, between January 2006 and January 2006, 196 courses of MTX infusion at medium dose of 1 gr/m^2/36 hours were evaluated. Subjects with wild type (CC), heterozygote, and homozygote MTHFR polymorphism was 47 (55. 3%), 29 (34. 1%), and 9 (10. 6%), respectively. The distribution of wild type (CC), heterozygote, and homozygote MTHFR polymorphism for the MTX infusion group was found to be 100 (51. 0%), 80 (40. 8%), and 16 (8. 2%), respectively. We did not found any effect of MTHFR polymorphism for the prognosis and outcome of the patients. However, there is a correlation between MTHFR polymorphism and MTX related neutropenia and mucositis. **Summary and Conclusions.** Our data suggest that MTHFR gene variants play a critical role in MTX related mucositis and neutropenia in ALL patients. Further studies about the genotyping of folate pathway gene variants might be useful to enable reduction of chemotherapy toxicity and/or to improve survival by individualized dose adjustments or alternative treatments.

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TELOMERASE GENE SCREENING AND TELOMERE OVERHANG DETECTION IN CHINESE PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Telomeres are complex structures capping the ends of all eukaryotic cell chromosomes. Loss-of-function mutations in telomerase complex genes reduce telomerase activity and shorten overall telomere length in leucocytes, and they can clinically manifest as aplastic anaemia and dyskeratosis congenita, which predispose to acute myeloid leukemia. Telomeres are constituted of double-stranded tandem TTAGGG repeats followed by a 3' G-rich single-stranded overhang, a crucial telomeric structural component responsible for the t-loop formation. Loss of telomerase function also leads to short telometric overhangs, potentially resulting in chromosome instability. **Aims and Methods.** In this study, we screened bone marrow samples from 72 Chinese patients with acute myeloid leukemia (AML, excluding AML-M3) for variants in telomerase reverse transcriptase(TERT) and telomerase RNA component(TERC) gene. We also investigated the length of telomeric overhangs in those patients and 46 healthy individuals, using Southern blot analysis. Cytogenetic status, disease severity and short time survival rate in patients with AML were evaluated. **Results.** Our cohort included 2 M1,48 M2, 5 M4, 5 M5 and 12 secondary AML. Age was 48(13-77), medium follow-up time was 4 months (2-10 months). 3 TERT mutations (m896G>A□ m1079C>G and m1451G>A) but no TERC mutation was identified in patients with AML. Patients carrying TERT mutations had very short telomeres, critical short overhangs and poor response to the induction chemotherapy and died in their follow-up period. In contrast to overall telomere length, which shortened with ageing, telomeric overhang lengths were constant overtime and much shorter in patients with AML compared to those of normal controls(P<0. 001). In AML cohort, those who have shorter overhangs did worse than those with normal ones. Multivariant analysis showed telomeric overhang length, as well as unfavorable chromosome abnormalities, served as an independent prognostic marker for AML patients(P<0. 0001). **Conclusions.** We report 3 mutations in TERT gene patients with AML, which may cause short telomere and overhang and account for poor prognosis in those patients. Short overhang length may be an independent factor for poor response and shorter survival in patients with AML. These findings would have to be confirmed in large, prospective studies.

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CLOFARABINE FOLLOWED BY CYCLOPHOSPHAMIDE FOR TREATMENT OF RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULT PATIENTS

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Introduction. Relapsed or refractory adult acute lymphoblastic leukemias (ALL) have poor prognosis. The strategy for treating these patients is through reinduction chemotherapy followed by allogeneic stem cell transplantation, provided that the toxicity of the salvage regimen is acceptable. Clofarabine, a next-generation deoxyadenosine analog, has demonstrated significant activity in children and adults with refractory lymphoid and myeloid leukemia in early clinical trials and was granted approval for use in children with acute lymphoblastic leukemia in second or higher relapse. Promising activity of clofarabine in combination with cyclophosphamide, with DNA damage and apoptosis in both AML and ALL blasts, has been reported (Karp JE et al, Blood 2007). **Aims.** We present a series of ten cases in which clofarabine was combined with cyclophosphamidein adult patients with relapsed or refractory acute lymphoblastic leukemia. **Methods.** Patients aged 23-59 years with refractory/relapsedALL were treated at the dose of clofarabine 10mg/m2 + cyclophosphamide 400g/m2 on days 1-3 and 8-10. We evaluated the overall remission rate (ORR), duration of remission (DOR) and overall survival (OS). Minimal residual disease (MRD) by molecular targeting was considered in all patients. **Results.** Nine patients received clofarabine 10mg/m2 + cyclophosphamide400mg/m2, both on Days 1-5 and 8-10;one patient received only one cycle. All patients had relapsed/refractory lymphoblastic leukemia and had received multiple priors tharapies. Eight had pre-B cell ALL, 2 pts had T cell ALL; two pts had received a prior hematopoietic stem cell transplant (HSCT). Four patients achieved a complete remission (CR);two patients went on to receive allogeneic transplants after clofarabine/cyclophosphamide salvage. The median of Overal survival (OS) forall the patients was 103 days, the media was 172,70 days. The overall remission rate (ORR) was 44,4%, and we estimated a duration of remission (DOR) as 223,25 days in media (we calculated from the first day of remission). Treatment was complicated by neutropenic fever (n=4), grade III-IV mucositis (n=3), prolonged aplasia >30 days (n=3). One patient died of sepsis before completing the regimen. **Conclusions.** Combination treatment with clofarabine and cyclophosphamide in adults pts with refractory or relapsed ALL resulted inan ORR of 44%, two ptsproceeded to HSCT. The safety profile is acceptable in this relapsed/refractory population. The response rates and durability of remission observed with this regimen were encouraging given that these patients were highly refractory to prior therapies. However, more studies with this combination in adults are warranted.

Table 1.

N	Age	Diagnosis	Previous regimen (n.)	Response after Clofara-Cy	Toxicities	HSCT
1	35	B-ALL	3	Refractory	Neutropenic fever	n
2	57	B-ALL	4	Refractory	Neutropenic fever	n
3	52	B-ALL common	4+HSCT	Refractory	Prolonged marrow aplasia	n
4	23	pre T-ALL	4	Refractory	mucositis	n
5	27	B-ALL	4	Complete Remission	mucositis	y
6	59	B-ALL common	3	Complete Remission	Prolonged marrow aplasia	y
7	40	B-ALL	2+HSCT	Complete Remission	Neutropenic fever	n
8	57	T-ALL	2	Refractory	mucositis	n
9	57	B-ALL common	1	Complete Remission	Neutropenic fever	n
10	23	B-ALL	3	Refractory	Prolonged marrow aplasia	n

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ASSESTMENT OF NEUROPSYCHIATRIC LATE EFFECTS AMONG SURVIVORS OF CHILDHOOD LEUKEMIA

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Background. The cure rate has improved significantly with advances in the treatment of acute leukemia. As numbers of childhood leukemia survivors increase the importance of monitoring late effects become more important. **Aims.** To identify the neurophyschiatric late effects and to provide an assesment about the degree and incidence of late effects of acute leukemia treatment in children. **Patients and Methods.** Patients who had been diagnosed as acute leukemia at least five years ago and treated between 1993-2009, in Dokuz Eylül University Hospital Department of Pediatric Hematology were included. All of the patients were treated with BFM protocols and none of them had relapsed. Demographic features, signs of neurological and ophtalmological examination, cranial MR imaging (MRI), auditory tests and neurocognitive tests were performed. The control group for neurocognitive tests were consisted of sibling of the patient. Informed consent was obtained. Questionnaires of quality of life were filled in by patients or parents. **Results.** There were 44 patients whom were 8-31 years old (mean age 16. 4±6. 4 years). Of 44 acute leukemia survivors, 48% were females and 52% were males. 11% of the patients were diagnosed as AML and 89% of them were diagnosed as ALL. Patients that had been off therapy for 2-18 years, were diagnosed at least 5 years ago. One or more late effects detected by physical examination, neurological tests or neurocognitive tests encountered in 80% patients, and 64% patients specified at least one complaint in the quality of life questionnaire. Cataracts and/or increased intraorbital pressure were found in 9% of patients and sensorineuronal hearing loss was found in 9% of patients. MRI revealed pathological findings in 18% of patients. EEG abnormalities were present in 9% of patients. Evaluation of total intelligence scores revealed that 30% of patients' IQ scores were less than 80 and 70% of patients' scores demonstrated neurocognitive dysfunctions. Comparison of the neurocognitive functions between patients and the control group revealed no statistically significant difference. Patients who had central nervous system involment at the time of diagnosis had significantly more pathological findings on neurological examination and tests. Based on the BFM protocol risk criterias the patients in the high risk group at the time of the diagnosis had higher incidence of cataract. The patients > 18 years during the study, or < 6 years at the time of diagnosis, or had AML, or had taken high dose (18 Gy) radiotherapy had lower IQ scores. Also neurocognitive disorders were more frequently detected in the patients > 18 years at the time of study. The patients > 6 years at the time of diagnosis were found to have more psychological problems and higher rates of smoking and alcohol consumption. The most frequent complaint was headache and the most common problem in school was denoted as diffuculty in concentration. **Conclusions.** In our study at least one neuropyschiatric late effect was detected in most of the cases. Survivors of chilhood leukemia are at risk of developing neurological and neurocognitive late effects as a result of exposure to chemotherapy and radiotherapy.

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EARLY RISK OF OBESITY AND THE METABOLIC SYNDROME IN EGYPTIAN CHILDREN SURVIVING ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The metabolic syndrome is increasingly recognized in adults surviving childhood cancer . **Objectives.** This study aimed to assess the early prevalence of obesity and the metabolic syndrome in children surviving acute lymphoblastic leukemia(ALL). **Methods.** 33 ALL survivors of mean age 13. 8 ±4. 9 years, who completed therapy for a mean of 5. 2±2. 1 years were compared to 33 healthy controls. All patients received chemotherapy, 13 received prophylactic cranial irradiation . All were subjected to clinical history, examination, anthropometric measures; assessment of lipid profile, fasting blood glucose, serum insulin, serum uric acid, serum leptin, growth hormone level (after provocation test), thyroid hormone assays. **Results.** HDL cholesterol, HDL/total cholesterol were lower in survivors compared to controls(P < 0. 001),with no significant difference between the two groups of leukemia survivors. Among survivors, 81. 8% had low HDL and 39. 4% had hyperinsulinemia, 12. 1% were overweight,and 9. 1% were obese. Systolic blood pressure above the 90th centile for age and sex was observed in 2 patients (6. 1 %),associated with obesity in one patient,combined with overweight and hyperinsulinemia in one patient . None had frank hypertension. Only one male survivor (3%) fulfilled the main criteria of the metabolic syndrome (hyperinsulinemia, obesity,low HDL cholesterol, in addition to raised blood pressure). Among the remaining survivors, 11 (33. 3%) had low

HDL with hyperinsulinemia, 1(3%) had hyperinsulinemia and raised systolic blood pressure, 15 (45. 5%) had only low HDL. Overall, 28 out of 33 studied survivors (84. 8%) had at least one main risk factor of the metabolic syndrome . Growth hormone deficiency (GHD) was found in 15 survivors of ALL (45. 4%), with a significantly higher percentage (76. 9%) among survivors who had cranial radiotherapy (RT),compared to 25% in survivors who received chemotherapy only (p<0. 05) . Among the survivors with GHD,20%, (3 out of 15) were overweight and 20% (3 out of 15) were obese . However, among the non- GHD survivors 1 out of 18 was overweight (5. 5%), and none of them was obese, the difference was statistically significant (p<0. 05) . Thyroid hormones and serum leptin were normal in all leukemia survivors while serum uric acid was significanltly elevated compared to controls (p<0. 001) . Multivariate analysis identified increased systolic blood pressure as significant predictor for low HDL(p=0. 04), female gender and increased systolic blood pressure as significant predictors for hyperinsulinemia (p=0. 04 and p=0. 05,respectively), and increased BMI was significant predictor for combined low HDL with hyperinsulinemia (p=0. 02). **Conclusions.** Childhood leukemia survivors have early increased prevalence of obesity, astherogenic dyslipidemia and hyperinsulinemia, denoting early risk of metabolic syndrome. They need follow up for early detection and preventive intervention.

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MAGNETIC RESONANCE IMAGING OF THE BRAIN IN SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Delayed treatment-related neurological damage is becoming increasingly important now that more and more children survive ALL treatments. After modification of the treatment protocols, severe symptomatic late effects are rare, and most adverse effects are detected by sensitive imaging methods such as MRI or by neuropsychological testing. **Aims.** We aimed to evaluate the incidence and characteristics of late CNS damage by MRI and clinical examination in children treated for ALL at the oncology unit of Pediatrics department of Zagazig university hospitals. **Methods.** Our study included 25 patients who were consecutively enrolled and treated according to modified Children Cancer Group (CCG) 1991 protocol for standard risk ALL and modified CCG 1961 protocol for high risk ALL and who had survived more than 5 years from the diagnosis. All relevant data were collected from patients' medical records specially those concerning the initial clinical presentation and initial brain imaging. All patients were subjected to thorough history and full physical examination with special emphasis on neurological system. MR Imaging of the brain was performed for all patients. This study was done according to the international and local ethical standards and informed consents were obtained from all patients.

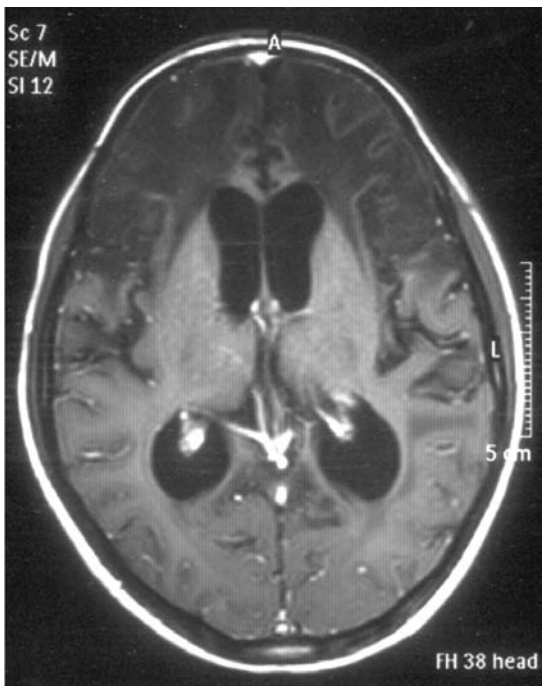

Figure 1. MRI brain of 13 year old boy previously treated with cranial irradiation displaying grade III leukoencephalopathy.

Results. Mean age of patients at diagnosis was 6. 9 ± 3. 04 and at study time was 12. 9 ± 3. 2. Patients were 14 males and 11 females. Abnormal MRI findings were detected in six patients (24%). They were in the form of leukoencephalopathy in two patients (8%), brain atrophy in two patients (8%), old infarct in one patient (4%) and old hemorrhage in one patient (4%). Abnormal MRI findings were significantly higher in high risk patients, patients who had CNS manifestations at diagnosis and patients who received cranial irradiation. **Summary and Conclusions.** We conclude that cranial irradiation is associated with higher incidence of MRI changes in children treated for ALL. Limitation of cranial irradiation to selected patients contributed to the lower incidence of neurological complications in our study. MRI is a sensitive radiological tool to detect structural changes in children treated for ALL even asymptomatic ones.

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WITHDRAWN

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THE INFLUENCE OF RANKL/OSTEOPROTEGERIN ON THE PROGNOSIS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The discovery of the nuclear factor-kappa B (RANK)/RANK ligand (RANKL)/ osteoprotegerin (OPG) system and identification of their role in osteoclastogenesis provided a major advance in bone biology. RANKL directly induces osteoclast differentiation and proliferation by binding to its receptor RANK on the surface of osteoclast precursors. OPG is the soluble decoy receptor for RANKL thereby inhibiting bone resorption. **Aims.** We aimed to investigate the association of the OPG/ soluble(s) RANKL with the prognosis of children with acute lymphoblastic leukemia. **Methods.** We investigated the relationship between OPG/sRANKL at the diagnosis and remission (at continuation phase) with the prognosis of children with acute lymphoblastic leukemia. The registration included demographic features (sex, age, risk group), nadir laboratory values [white blood cell count (WBC), lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), immunphenotype, involvement of central nervous system, bone mineral density at presentation], and data of response to the treatment, relapse and out-come. **Results.** Forty five children (20 girls and 25 boys; median age 72 months, range 22 -216 months) with ALL treated according to the Turkish national BFM ALL (TRALL) protocol at Erciyes University, Children's Hospital, Kayseri, Turkey, between February 2008 and February 2010 were enrolled to this prospective study. The distribution of standard risk group, medium risk group, high risk group according to the TRALL was 14 (31. 1%), 20 (44. 4%), 11 (24. 4%) respectively. During the study 6 patients showed relapse, moreover 6 patients died because of progressive disease and 2 children died because of infection. The serum levels of OPG and RANKL at diagnosis were 58. 29±86 ng/ml, 0,29±0. 41 ng/ml, respectively; whereas their values at remission were 35. 5±40. 86 ng/ml, 0,03±0. 05 ng/ml, respectively. We detected a significant decrease for OPG (p=0. 000) but the decrease for sRANKL was not below the level of significance (p=0. 699). There was a correlation between initial levels of OPG, sRANKL and initial WBC, LDH. But we did not found any correlation with prognosis (response to treatment, relapse and out-come). **Summary and Conclusions.** Our data suggest that OPG and sRANKL is increased at diagnosis of childhood ALL. We speculate that, it may be related to the micro environment of the initial leukemic cell burden. Additionally we did not found any relation with prognosis. Further studies such as flow-cytometric expression with large numbers are necessary to highlight the clear effect of these molecules in children with ALL.

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BACTEREMIA AND PATTERNS OF BACTERIAL SUSCEPTIBILITY AMONG CHILDREN WITH HEMATOGICAL MALIGNANCIES

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Background. Infections cause substantial morbidity and morbidity in children with hematological malignancies receiving chemotherapy. Epidemiology of bac-

teremia differs between institutions and constitutes the basis for selection of empiric antibiotic therapy for febrile neutropenia. **Aims.** To investigate the long-term epidemiology and resistance of pathogens isolated from blood cultures in children with hematological malignancies. **Patients and Methods.** We conducted a retrospective data collection on all positive blood cultures from children with hematological malignancies in a pediatric Oncology Department during 2002-2011. **Results.** A total of 225 bacteremic episodes and 2 candidemias were documented in 136 children with median age of 6 years old (range 1-16) and 42% boys. The majority of patients (69. 8%) was diagnosed with acute lymphoblastic leukemia, 20. 5% (28/136) with non-Hodgkin lymphoma, and 9. 5% (13/136) with acute myeloid leukemia. The leading pathogen was Coagulase-Negative-Staphylococci (CNS) (49. 3%), followed by *Klebsiella* spp. (10. 6%), *Pseudomonas* spp. (8. 9%), *Escherichia coli* (8. 4%), *Enterobacter cloacae* (3. 1%) and *Staphylococcus aureus* (2%). It is noteworthy that 48% of *Klebsiella* spp. and 50% of *E. coli* isolates were extended beta-lactamase producers (ESBL), while 8% of *Klebsiella pneumonia* but none of the *E. coli* isolates were resistant to imipenem. 20% of the *Pseudomonas aeruginosa* isolates were resistant to ceftazidime. Vancomycin resistance was noted in 1% of the CNS, but none of the *Staphylococcus aureus* isolates. CNS and *S. aureus* were 80% and 9% resistant to methicillin (MRSA), respectively. Since the beginning of the study Gram-negative episodes increased (from 3. 1 to 13. 7/1000 patients, chi-square for trend p<0. 01) and Gram-positive episodes decreased from 18. 14 to 8/1000 patients, chi-square for trend p<0. 01). The overall mortality was 22. 7% while infection related mortality was 8. 8%. **Conclusions.** Gram-positive bacteria are the more commonly isolated etiologic pathogens but the significant increase of Gram-negative episodes needs special attention in children with hematological malignancies. Infection epidemiology among patients with hematological malignancies should be monitored in order to guide prompt empiric antibiotic treatment.

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OUTCOME OF PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA- A SINGLE CENTRE EXPERIENCE

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Background. Philadelphia chromosome-positive (Ph+) Acute Lymphoblastic Leukemia accounts for about a quarter of adult ALL. This group of patients is associated with dismal outcome with standard chemotherapy. In recent years, haematopoietic stem cell transplantation (SCT) has been an attractive curative option to improve survival in these patients. Patients in our centre are treated with combination chemotherapy with tyrosine kinase inhibitors as induction therapy prior to SCT. **Aims.** We evaluate the outcome of patients with Ph+ Acute Lymphoblastic Leukemia who had undergone combination chemotherapy versus haematopoietic stem cell transplantation. **Methods.** All records of the patients were analyzed in a non-randomized retrospective study on 1st February 2012. Overall survival was estimated using the Kaplan-Meier method. **Results.** There were 35 patients, diagnosed with Ph+ ALL out of 141 patients with B lineage ALL (24. 8%) from January 2007 to Dec 2011. The median age of patients at diagnosis was 36 years (range 22 to 62). Male to female ratio was almost 1:1 with an ethnic composition of Chinese 48. 6 %, Malays 34. 3 %, Indian 11. 4 % and others 5. 7 %. In our cohort, 21 patients were unable to undergo stem cell transplantation due to refractory disease, medically unfit or no HLA-matched donor. In this group of patients, only 18 patients were analyzed as 3 were lost to follow-up. A total of 14 patients (40%) underwent haematopoietic stem cell transplantation (9 HLA-identical sibling, 4 unrelated HLA-matched donor, 1 autologous). Four-year overall survival (OS) for patients who underwent haematopoietic transplantation versus patients without transplantation were 69. 6 % and 0 % (p= 0. 0001). Median follow-up for these two groups was 22. 5 months and 7 months respectively. Eleven out of 14 patients were in first complete remission (CR 1) prior to transplantation. Amongst the 9 HLA-identical siblings allo-SCT, three died due to disease progression and/or veno-occlusive disease. There was one mortality due to GVHD in the unrelated HLA-matched allo-SCT group. The patient with autologous transplantation is still in complete remission. All patients are monitored for minimal residual disease (MRD) by PCR real time quantitative. **Conclusions.** The analysis of a small cohort of our patients with Ph+ ALL suggests a trend towards a favorable outcome with HSCT. Statistical inferences are limited by small number of patients, short duration of follow-up and non-randomization. Longer duration of follow-up and a prospective randomized comparative trial is needed to conclusively confirm the finding.

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OUTCOME OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED BY THE EORTC 58951 PROTOCOL ABOUT 152 CASES

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Background. Acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in children, representing nearly one third of all pediatric cancers. In this study we analysed the clinical, biological features and therapeutic results of patients treated for ALL and aged less than 19 years. **Patients and Methods.** From January 2000 to December 2010, 152 patients aged from 13 months to 19 years were treated for ALL by the pediatric EORTC 58951 protocol in the Hematologic department of Hedi Chaker Hospital. From these patients, we analysed the clinical and biological features (Age, sex, blood count, cytogenetics abnormalities and blasts phenotypes) and therapeutic results (remission, relapse, overall survival (OS), event free survival (EFS) and relapse free survival (RFS) at 5 years or follow up). **Results.** The median age of patients was 9 years. Sex ratio H/F= 1. 45. The median WBC count at diagnosis was 19500 cells per µL (ranged from 700 to 402000 cells per µL). The high leucocytosis more than 100000 cells per µL was observed in 17. 5% children. There were 66% with B-cell ALL and 34% with T-cell ALL. Among 152 children, 135 (89%) have a successful cytogenetic result: normal karyotype, hyperdiplopid >50 chromosomes, t (9, 22) and del 11q23 translocation were found respectively 49%, 33%, 7% and 6%. The Cortico-resistance at day 8 of corticosteroid (more than 1000 blasts/mm^3 on the blood smear) was observed in 17% of patients. Poor prednisone response was associated with high leucocytosis and T-cell ALL ($p<10^{-4}$). The children were stratified into low risk, average risk and high risk in respectively 4% (6 patients), 70% (106 patients) and 27% (40 patients). Seven children (4. 5%) were treatment failure after two courses of chemotherapy and 13 patients (8. 5%) died during induction. The complete remission was obtained in 133 children (95%), among them 29 relapsed (22%). The death of post induction was noted in 7% patients. At 5 years of follow-up, the OS, EFS and the RFS were respectively 60%, 57% and 71%. **Discussions.** Our study is characterized by higher rate of male gender and T phenotype of blasts than reported in the literature. Despite the acceptable results concerning remission rate and survival, we have a high rate of death related to chemotherapy. Therefore, the need to improve supportive treatment in the management of our patients

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HYPEREOSINOPHILIA AS FIRST CLINICAL PRESENTATION OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: REPORT OF TWO SUBSEQUENT CASES

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Background. Hypereosinophilia as first clinical presentation has rarely been reported in pediatric acute lymphoblastic leukemia (ALL). This subtype of leukemia is frequently associated with specific cytogenetic abnormalities, even if eosinophilia seems to be a reactive, nonneoplastic epiphenomenon. Hypereosinophilia seems to affect negatively the prognosis of this unusual ALL. **Aims.** On a cohort of 340 ALL children, treated with a protocol in a single pediatric institution, we observed two consecutive patients with marked eosinophilia as the main clinical onset of ALL. The coincidence of two subsequent ALL with hypereosinophilia at presentation warranted a description of their case histories. **Case 1.** A 13-year-old-girl with history of evening feveret and fatigue. Blood examination: normal leukocyte count associated with hypereosinophilia (44. 3%), mild anemia and thrombocytopenia. Peripheral blood smears: morphologically mature eosinophils and absence of blasts. No parasitic or allergic conditions were found. Bone marrow aspirate: marked eosinophilia (30%) and partial infiltration of L2 lymphoblasts (52%). Immunophenotyping: B-lymphoid CD10 positive blasts, and the cytogenetic analysis showed a 46, XX karyotype; the main molecular leukemic rearrangements (bcr/abl, TEL/AML1, MLL/AF4, E2A-Pbx1) were negative, including Jak2 and 4q(12). Doppler ultrasound: left iliac-femoral deep venous thrombosis as thrombotic complication. Treatment: AIEOP-BFM ALL 2000 protocol, without L-Asparaginase, for its pro-thrombotic property. Outcome: complete hematological remission during maintenance treatment 8 months from diagnosis. **Case 2.** A 20 month-old-boy, fifteen days after the first patient,

experienced mild leukocytosis, hypereosinophilia (32. 1%), feveret, night sweat, pruritic erythroderma and left inferior limb arthralgia with claudication; all other hematological parameters were normal and no liver or spleen enlargement was observed (Table 1). Peripheral blood smears: eosinophils associated with lymphoblasts (11%). Bone marrow aspirate: diffuse infiltrates (60%) of L1 CALLA positive lymphoblasts and hypereosinophilia (36%). Skin biopsy of the erythroderma confirmed cutaneous infiltration by leukemic cells associated with eosinophilia. CT scan of the left inferior limb: tibial osteolytic area with interruption of cortical bone; the biopsy of this area showed leukemic and eosinophilic infiltration. Cytogenetic studies: hyperdiploid karyotype (52 chromosomes), with all leukemic rearrangements negative, including bcr/abl, and 4q(12). Treatment: AIEOP-BFM ALL 2000 protocol. Outcome: hematological complete remission during reinduction at 7 months from diagnosis. The short follow-up of both patients does not allow any prognostic evaluation. **Comments**. Although, in our experience, the incidence of hypereosinophila, as first clinical presentation of ALL, was very rare (0,6%), we emphasized the importance of a correct differential diagnosis in persistent, unexplained peripheral hypereosinophilia. Clinicians should keep in mind that eosinophilia can be caused by acute leukemia and needs to be explored with a bone marrow aspirate. The poor prognosis reported for these atypical ALL in children should prompt clinicians to carry out an accurate and prolonged follow-up. Besides, childhood ALL with hypereosinophilia requires careful surveillance, for an early recognition and prompt treatment of eosinophilia-related morbidities, such as cardiac failure, pulmonary pathology, peripheral neuropathy and thromboembolic phenomena.

Table 1. Patients' clinical characteristics.

Characteristics	Patient 1	Patient 2
Sex	Female	Male
Age at diagnosis	13 years	20 months
Peripheral blood:		
Hb(g/dL)	9,8	12
White blood cells count (mL)	7870	14390
Eosinophil count (mL)(%)	3490 (44.3%)	4620 (32.1%)
Platelet count (mL)	129.000	223.000
Blasts (%)	None	11
Bone marrow aspirate:		
Blasts (%)	52	60
Eosinophils (%)	30	36
FAB category	L2	L1
Immunophenotype	Common	Common
DNA index	1	1.01
Bone marrow biopsy	B cells lymphoproliferative syndrome; MF1 (WHO grading)	B cells lymphoproliferative syndrome; MF0 (WHO grading)
Karyotype	46,XX	Hyperdiploid (52,XXY,+6,+14,+17,+21,+21[5])
Molecular biology for ALL rearrangements	Negative	Negative
Molecular biology for thrombophilia	Positive (Homozygous for -455G/A of b-fibrinogen)	Positive (Eterozygous for P1b of HPA)
Clinical presentation	Hypereosinophilia Evening feveret Left iliac-femoral deep venous thrombosis	Hypereosinophilia Claudication Slight fever Night sweat Left inferior limb arthralgia Pruritic erythroderma
Other organ involvement	No	Skin Bone (tibial osteolysis)

FAB, French-American-British classification; MF, myelofibrosis; WHO, World Health Organization; HPA, human platelet antigen

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STUDY OF VON WILLEBRAND FACTOR AND FACTOR VIII LEVELS IN CHILDREN WITH NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA IN RELATION TO PERIPHERAL BLAST CELLS AND STEROID THERAPY

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Background. The pathogenesis and the impact of therapy on thrombin activation in children with acute lymphoblastic leukemia (ALL) are unknown. Steroids may contribute to ALL-associated thrombosis. **Aims.** Of this study is to assess children with newly diagnosed ALL for Von Willebrand Factor antigen and Factor VIII relevant to peripheral blast cells and the effect of steroids therapy on their levels. **Methods.** This study was conducted on 32 newly diagnosed patients ALL patients; 24 patients with peripheral blasts, and 8 patients without. **Results.** The study showed that at diagnosis patients with peripheral blasts had a significantly higher levels of VWF antigen, FVIII, and total leucocytic count than those without. After 8 days of steroid therapy, patients with peripheral blasts had a significant reduction in both factors, while those without peripheral blasts showed a significant increase in VWF antigen, and FVIII. There was a significant positive correlation between total leucocytic count and VWF antigen, and between peripheral blasts and VWF antigen, FVIII at diagnosis among ALL patients with peripheral blasts. **Conclusions.** In presence of circulating blasts, steroids have two types of effects: a direct effect resulting in the increase in VWF antigen and FVIII levels and an indirect effect through reduction in circulating blasts which results in normalization of endothelium and resultant reduction in both factors levels. This reduction may be in excess of steroid-induced increment in VWF antigen and FVIII levels with a net effect of reduction in the levels of both.

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OUTCOME OF CORTICORESITANT PATIENTS TREATED WITH THE PEDIATRIC EORTC 58951 PROTOCOL FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Corticoresistance at day 8 has been described as one of high risk factor in acute lymphoblastic leukaemia (ALL). In this study we analysed the impact of corticoresistance on the prognosis of patients treated for ALL. **Patients and Methods.** Between January 2000 and December 2010,175 patients were treated for ALL with the pediatric EORTC protocol in the hematologic department of Hedi Chaker hospital. Risk group stratification was made according to White blood cells count, blasts phenotype, cytogenetics abnormalities, response to corticosteroids (Préphase) at day 8 and response to chemotherapy in the end of induction. High risk group is considered if one of these criteria was observed: Cyto-genetics abnormalities like t(9,22) or del11q23, Cortico-resistance at day 8 of Préphase (more than 1000 blasts/mm^3 on the blood smear) and chemo-resistance at the end of induction. We studied the remission rate, the 5 years relapse free survival (RFS) and the 5 years overall survival (OS) for cortico-resistant patients . Then we compared these results to those of corticosensitive patients. **Results.** Among 175 ALL patients aged from 1 to 28 years and treated by the pediatric EORTC 58951 protocol, 31were corticoresistant at day 8 of Préphase (18%). Corticoresistance was the only high risk factor in 22 patients (71%). The rate of death during induction and post induction therapy were respectively 18% and 10% for corticoresistant and corticosensitive ALL. The remission rate was 84% for corticoresistant ALL and 98% for corticosensitive ALL $P<10^{-4}$. Among 24 corticoresitant patients allograft was indicated for 22 and only six patients undergone allograft with a familial donor. At 5 years of follow up, the RFS and the OS were respectively 42% and 23% for corticoresistant compared to 78% and 68% for cortico-sensitive ALL ($P= 0.0006$ and $P<10^{-4}$) **Conclusions.** In this study, corticoresiatnce was more observed than described in the literature 18% vs 9%. And it has a negative impact alone or with the other high risk factors on the remission rate and in the outcome of the ALL (RFS and OS). Allograft should improve the outcome of majority of patients and we think that the minimal residual disease study recently introduced in the follow-up of our patients can distinct some cortico-resistant ALL with better prognosis.

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THE INFLUENCE OF CD40/CD40 LIGAND ON THE PROGNOSIS OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. CD40 plays a critical role in immunoregulation, the levels of sCD40 in adult patients with hematologic malignancies were reported to be elevated, and associated with a poor prognosis at least in patients with MM and AML, suggesting that sCD40 may have a role in modulating antitumor responses and also may be a useful prognostic marker. Experience in children, however, is extremely limited. **Aims.** We aimed to investigate the association of the CD40/CD40 ligand with the prognosis of children with acute lymphoblastic leukemia (ALL). **Methods.** We investigated the relationship between CD40/CD40 ligand at the diagnosis and remission (at continuation phase) with the prognosis of children with acute lymphoblastic leukemia. The registration included demographic features (sex, age, risk group), nadir laboratory values [white blood cell count (WBC), lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), immunophenotype, involvement of central nervous system, bone mineral density at presentation], and data of response to the treatment, relapse and out-come. **Results.** Forty five children (36 B cell, 9 T cell; 20 girls and 25 boys; median age 72 months, range 22 -216 months) with ALL treated according to the Turkish national BFM ALL (TRALL) protocol at Erciyes University, Children's Hospital, Kayseri, Turkey, between February 2008 and February 2010 were enrolled to this prospective study. The distribution of standard risk group, medium risk group, high risk group according to the TRALL was 14 (31. 1 %), 20 (44. 4%), 11 (24. 4%) respectively. During the study 6 patients showed relapse, moreover 6 patients died because of progressive disease and 2 children died because of infection. The serum levels of CD40/CD40 ligand at diagnosis were 22. 41±9. 91 ng/ml, 0. 22±0. 38 ng/ml, respectively; whereas their values at remission were 15. 17±5. 49 ng/ml, 1. 04±0. 51 ng/ml, respectively. We detected a significant decrease for CD40/CD40 ligand (p=0. 008 and 0. 000 respectively). There was a correlation between initial levels of CD40 and initial WBC, LDH, immunophenotyping, CNS involvement, But we did not

found any correlation with prognosis (response to treatment, relapse and outcome). **Summary and Conclusions.** Our data suggest that CD40/CD40 ligand is increased at diagnosis of childhood ALL. On the bases of its physiological effect CD40 may have a role in modulating antitumor responses and also may be a useful prognostic marker. Additionally we did not found any relation with prognosis. Further studies such as flow-cytometric expression with large numbers are necessary to highlight the clear effect of these molecules in children with ALL.

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THE EFFECT OF MTHFR POLYMORPHISMS ON THE PROGNOSIS AND SEVERITY OF ORAL METHOTREXATE RELATED TOXICITY DURING CONTINUATION THERAPY IN CHILDREN WITH ALL

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Background. Methotrexate (MTX), which interrupts folate metabolism, is the fundamental drug widely used in the treatment of childhood acute lymphoblastic leukemia (ALL). Methylenetetrahydrofolate reductase **(**MTHFR) is the most important enzyme in this pathway and has a wide polymorphism. **Aims.** We aimed to investigate the association of the MTHFR gene (C677T) polymorphisms with the prognosis and severity of oral methotrexate related toxicity during continuation therapy in children with acute lymphoblastic leukemia. **Methods.** We investigated the relationship between MTX related neutropenia and mucositis and MTHFR polymorphism. Toxicities were assessed using the Common Terminology Criteria for Adverse Events (CTCAE), Version 3. 0, published by the National Cancer Institute. The registration included nadir laboratory values [white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin level (Hb), platelet count (PLT), maximum plasma values of alanine aminotransferase (ALT)], and delay in the chemotherapy regimen. **Results.** Eighty five children (54 boys and 31 girls; mean age 6. 3±3. 6, range 1-16 year) with ALL treated with oral 6 mercaptopurine 40 mg/m2/day and methotrexate 25 mg/m2/week for the continuation therapy according to the Turkish national BFM ALL protocol at Erciyes University, Children's Hospital, Kayseri, Turkey, between January 2006 and January 2006 were enrolled to this retrospective study. Subjects with wild type (CC), heterozygote, and homozygote MTHFR polymorphism was 47 (55. 3%), 29 (34. 1%), and 9 (10. 6%), respectively. The distribution of wild type (CC), heterozygote, and homozygote MTHFR polymorphism for the MTX infusion group was found to be 100 (51. 0%), 80 (40. 8%), and 16 (8. 2%), respectively. We did not found any effect of MTHFR polymorphism for the prognosis and outcome of the patients. However, there is a correlation between MTHFR polymorphism and MTX related neutropenia and mucositis. **Summary and Conclusions.** Our data suggest that MTHFR gene variants play a critical role in MTX related mucositis and neutropenia in ALL patients at the continuation phase. Further studies about the pharmacogenetic area are necessary to enable reduction of chemotherapy toxicity with individualized dose adjustments.

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ACUTE METABOLIC SIDE EFFECTS OF REMISSION INDUCTION TREATMENT IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Remission-induction treatment phase in children with ALL is critical due to the fact that severe complications like tumor-lysis syndrome might occur. It is important to know possible metabolic derangements and take the precautions for reducing additional morbidity and mortality. **Aims.** The aims of this study is to report the metabolic abnormalities during remission induction treatment, to evaluate the complications and define their effects on treatment process. **Methods.** 40 ALL patients (0-15 years old) in the remission-induction phase treatment were included to the study. TRALL BFM 2000 treatment protocol was used on the patients. In every patient alkaline hydration and allopurinol treatment for TLS prophylaxis were starting before ALL protocol. Baseline complete blood count, biochemical parameters, PT and aPTT were measured . On follow-up, deviations from normal parameters were recorded according to the protocol day. The frequencies of these deviations, possible aetiological causes, durations and clinical reflections were recorded. The patients, in whom the treatment was postponed due to metabolic abnormalities and their 33rd day responses were evaluated. **Results.** During the remission-induction phase, at least one metabolic disorder was detected 97. 5%. Increase in urea level was found to be the most common metabolic disorder (62. 5%). These were followed by hypophosphatemia and hypocalcemia (%52,5) hyponatremia (50%) hyperuricemia (27. 5%), hypochloremia (25%), hypopotassemia and hyperphosphatemia (22. 5%), hyperpotassemia (10%), hypomagnesemia and hypermagnesemia (7,5%). Transient hyperglycemia was detected 5%, hypertriglyceridemia 20%, hypertransaminasemia 52. 5% and hyperbilirubinemia 35%. LTLS rate was determined as 5% and KTLS was 2. 5%. In none of the cases indication for dialysis and mortality due to TLS were encountered. **Conclusions.** Remission-induction treatment in childhood ALL is critical due to the fact that severe complications like tumor lysis syndrome might occur. Allopurinol and alkaline hydration therapy were found to be succesfull in TLS prophylaxis due to the low mortality and morbidity rates.

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ALEUKEMIC LEUKEMIA CUTIS: AN UNUSUAL RASH IN A CHILD

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Background. Leukemia cutis is an infiltration of the skin by neoplastic leukocytes (myeloid or lymphoid), resulting in clinically identifiable cutaneous lesions. It is seen most commonly in congenital leukemia and acute myelogenous leukemia (approximately 10%). In the pediatric population, the frequency of leukemia cutis in acute lymphoblastic leukemia is rare (1%). **Aims.** Although leukemia cutis tends to present with other features of leukemia, it can occasionally precede the development of blast cells in the marrow and blood (aleukemic leukemia cutis). The aim of this case presentation is to illustrate how leukemia cutis can masquerade as a clinically benign-appearing cutaneous eruption without leukemic changes in blood or bone marrow. To confirm the diagnosis immunohistochemistry of the skin lesions as well as a complete staging procedure is necessary. **Case Report.** A previously healthy twenty two months old girl presented with a sudden onset rash consisting of erythematous macules on an abdomen. Over the next two week, the rash became papular and darker. The patient had no other symptoms or signs. Complete blood count showed no leukocytosis (WBC: 5. $95x10^9$/L) and thrombocytopenia (Plt: $215x10^9$/L) but anemia (Hb: 8. 46 g/dL). Biochemical analysis revealed high lactate dehydrogenase level (LDH: 873 U/L) and peripheral blood smear showed no blast cells. A skin biopsy performed four weeks after the onset of the rash showed leukemic blast cells (precursor B cell lymphoblastic lymphoma/leukemia). A bone marrow aspirate confirmed precursor B-cell lymphoblastic leukemia with 30% blast cells. **Conclusions.** Leukemia cutis can be the first sign of leukemia, preceding the diagnosis of systemic leukemia by months or even years. A sudden, persistent rash of unknown cause warrants full physical examination, hematologic investigation including complete blood count and dermatological evaluation. Remission can achieved with early recognition of leukemia cutis and prompt hematological treatment in aleukemic patients.

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EFFECTS OF RECOMBINANT URATE OXYDASE (RASBURICASE) AND ALLOPURINOL FOR PROPHYLAXIS AND TREATMENT OF HYPERURICEMIA IN PATIENTS WITH LEUKEMIA AND LYMPHOMA

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Background. Efficacy of rasburicase in pediatric patients with leukemia and lymphoma is proved. This study aims to weigh efficacy and safety of rasburicase versus more conventional therapy, allopurinol, and to compare their safety and properties in tumor lysis syndrome (TLS) of leukemia and lymphoma patients. Materials and **Methods.** The study was done with a retrospective cohort design. Patients were selected from our hematology ward admitted from 2005 through 2008. Patients were put into two groups based on their blood levels of uric acid, before initiation of chemotherapy; treatment group (the Uric Acid level of 6. 5 mg/dl or more) and the prophylaxis group (the uric acid level below 6. 5 mg/dl). Evaluation of effectiveness of therapy was performed after 24, 48, 72-hour, and longer periods. **Results.** Of 184 patients; 69% had leukemia, and 31% lymphoma. Twenty patients were treated with rasburicase and 164 with allopurinol. Mean age of patients was 7. 93± 4. 247 years old. 60. 8% were male and 39. 2% were female. According to Chi-square test results, there was no significant difference between two agents regarding prophylaxis (chi-square =

4. 247, p-value = 0. 193) and treatment (chi-square = 0. 780, p-value = 0. 677). Most of the response to each agent was seen in the first 24 hours after drug administration. Mean level of uric acid reduced from 7. 4 to 3. 4 mg/dl in rasburicase, and from 5. 4 to 3. 9 mg/dl in allopurinol group. Mean duration of treatment for rasburicase was 2 days, and for allopurinol 6 days. Adverse effects were minimal in both groups (in rasburicase 1. 6% and in allopurinol 5. 4%). **Conclusions.** Rasburicase seems to be highly efficient in both prophylaxis and treatment of Hyperuricemia. Due to high costs in our practice, it was only administered to 20 patients with high levels of blood uric acid or leukocytosis. It prepares patients for chemotherapy faster and decreases cost of hospital stay indirectly by lowering cost of treatment. Allopurinol, alternatively showed equal efficiency and comparable results. Thus, it can be used safely and effectively until rasburicase becomes more widely available and more cost-effective.

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THE BONE MARROW AND THE BONE SURFACE CELLS IRRADIATION DOSES IN CHILDREN WITH ACUTE LEUKAEMIAS WHICH WERE EXPOSED TO IONIZING RADIATION DUE TO CHERNOBYL ACCIDENT

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Background. Small doses of radiation can promote development of genetic and cancerogenic effects, in particular the development of acute leukemias in children's population which have suffered in result of Chernobyl accident (CA). **Aims.** To study the influence of small doses irradiation of the bone marrow (BM) and the bone surface cells (BSC) after Chernobyl accident on development of acute leukaemias (AL) in children and duration of their life. **Methods.** 19 children with AL are surveyed. Sex, age at the diagnosis, life expectancy of children (less than 60 months and more than 60 months), time passed from CA was considered. The accumulated irradiation doses for BM and the BCS resulting from internal acting of radionuclides are calculated, results of studying ^{137}Cs and ^{90}Sr dynamics of entering and radionuclides content in child organism are used and age features of consumption of local manufacture provisions are considered. **Results.** AL children age was in 2 -16 years limit. 13 children had ALL, 6 - AML. 13 children had life expectancy more than 60 months, 6 - less than 60 months. Equivalent BM irradiation doses of children fluctuated from 0,08 to 35,02 mZv, and BSC irradiation doses - from 0,11 to 35,41 mZv. The maximum doses had inhabitants of Chygyry village in Korostensky district of Zhitomir region that is caused by considerable levels of soils pollution (density of ^{137}Cs pollution is 492 kBq·м$^{-2}$, and ^{90}Sr - 9,25 kBq·м$^{-2}$) and permanent living in radioactive contaminated territory. The average irradiation dose for BM in this children group was 5,37 mZ, and for BSC - 5,58 mZv. **Conclusions.** There were no correlation between AL development, irradiation dose, age, sex and the life expectancy of children revealed. Direct correlation between children age and the accumulated doses from internal irradiation of BM and BSC is established (Ro-Spearm. = 0,46 and Ro-Spearm. = 0,49 accordingly).

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SURVIVIN EXPRESSION IN ELDERLY AML PATIENTS AT DIAGNOSIS AND DURING HEMATOLOGICAL REMISSION AFTER CHEMOTHERAPY

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Background. AML is a complex disease with considerable phenotypical as well as genotypical heterogeneity, and more than 50% of AML patients are over 60 years of age. Increasing evidence indicates that the unique member of the inhibitors of apoptosis proteins (IAPs), survivin, is implicated in regulation of cell division, apoptosis inhibition and drug/radiation resistance in various cancer cells. Many observations also provide new perspectives on survivin, as a valid therapeutic target for treatment of cancer and suggest new research directions for therapies without toxicity to normal human tissues· a process that is very interesting for adult AML patients. **Aims.** The Aim of this study is to evaluate survivin expression in elderly AML patients at diagnosis and during hematological remission after chemotherapy and correlate it with the expression in control individuals. Furthermore, survivin expression -in both mRNA and serum protein level- will be correlated with well-established AML prognostic factors and patients' response to chemotherapy. **Methods** Total RNA was isolated from peripheral blood cells (PBCs) of 40 elderly patients with AML. All patients (24 males and 16 females) were above 60 years of age (range 60-86 years, median age 67,72±11,47 years) and had not been treated prior to the study. Diagnosis was based on standard clinical and laboratory criteria. All patients were treated on standard AML protocols, with Ara-C and Idarubicin and 18 of them (10 males and 8 females) succeeded hematological remission (bone marrow blasts <5%) and were investigated again, during remission. Twenty-five healthy individuals, with normal haematopoiesis, age and sex matched, consisted the control group. Real-Time PCR assay was performed for the quantification of survivin mRNA levels. All PCR reactions were carried out in an Opticon2 Real-Time PCR System. Relative quantification of survivin mRNA expression was performed by employing the relative Ct method. Data was analyzed using the REST-XL©-Version2 software. Serum survivin protein levels were measured in patients at presentation and during remission, as well as in control individuals, using the ELISA assay. Statistical analysis was performed using the SPSS11. 5 software. **Results.** The mean survivin mRNA levels of PBCs in patients at presentation were significantly higher by 2,562 times (p=0,038), related to mean mRNA levels of patients in remission and significantly higher by 5,672 times (p=0,042) compared to control group. Serum survivin protein concentration was detected in all patients (100%) at diagnosis and in 11 out of 18 patients (61,2%) in hematological remission. The mean serum survivin protein concentration was 33,19±24,89 pg/ml in patients at diagnosis, while during remission detectable levels were found, although very low (0,011±0,028 pg/ml) (p=0,005). Serum survivin protein was undetectable in control individuals. High survivin mRNA levels were significantly correlated with unfavourable karyotype (p=0,033) and failure to response to chemotherapy (p=0,037), while high survivin serum levels were correlated with patients' failure to achieve remission (p=0,036). **Conclusions.** In our study both mRNA and serum protein levels of survivin were down-regulated after chemotherapy and were significantly correlated with failure to achieve remission. Thus, survivin could be used as an unfavourable prognostic marker or a marker of resistance in chemotherapy.

1199

BAALC CAN PROMOTE THE SURVIVAL AND INHIBIT THE AS2O3-INDUCED APOPTOSIS IN HL60/DOX-BAALC THROUGH BCL-2 AND NF-κB P65 UP-REQULATION SIGNALING PATHWAYS BUT NOT IN MOLT4-BAALC

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Background. BAALC (brain and acute leukemia, cytoplasmic) located at chromosome 8q22. 3, normally is highly conserved in neuroectoderm-derived tissues among mammals and CD34-positive progenitor cells from bone marrow, but it can't be found in lower organisms and mature hematopoietic cells. Under pathological conditions, BAALC overexpression can be found in patients with AML, ALL, and chronic myelogenous leukemia in blast crisis, which leads to a poor prognosis. Although it is confirmed that BAALC is correlated with the occurrence of AL, the biological function of BAALC protein at the molecular level still largely unknown. **Aims.** This study aims to investigate the biological function and the molecular mechanism of BAALC in AL. **Methods.** Molt-4 (human T-cell lymphocytic leukemia cell) and HL60/Dox (human doxorubicin resistant promyelocytic leukemia cell) whose have no original BAALC expression were chosen as the representative cells of T-ALL and AML respectively. BAALC 1-6-8 isoform was introduced into the Molt-4 and HL60/Dox cells with retrovirus infection. The stable BAALC overexpression in the two cells after the infection was confirmed by both quantitative real time PCR and western blotting. Survival rate of HL60/Dox-BAALC, Molt-4-BAALC and negative control were Analysis by MTT assay. As_2O_3-induced apoptosis of HL60/Dox-BAALC, Molt-4-BAALC and negative control were assessment by using Annexin V-FITC/PI apoptosis kit. The expression level of MDR1 was detected by using quantitative real-time PCR amplification and the he expressions of BCL-2 and NF-κB p65 were detected by western blotting. **Results.** MTT and apoptosis assays showed that HL60/Dox-BAALC had a significantly higher survival rate (P<0. 001) and a much lower As_2O_3-induced apoptosis rate (P<0. 001) than those of HL60/Dox and HL60/Dox-Vector; BAALC overexpression had no such effects on Molt-4-BAALC (P>0. 05). RT-PCR showed that the expression level of MDR1 in HL60/Dox-BAALC has no statistical difference (P>0. 05) compared with that in HL60/Dox and HL60/Dox-Vector. Western blotting showed that both BCL-2 and Nuclear factor κB (NF-κB) p65 were activated in the As_2O_3-induced apoptosis of HL60/Dox-BAALC. **Conclusions.** BAALC can affect the survival rate and As_2O_3-induced apoptosis of HL60/Dox-BAALC through BCL-2 and NF-κB p65 up-regulation signaling pathways, but the effects did not occur in Molt-4-BAALC.

1200

FLT3-ITD MUTATION DIFFERENTIALLY PRESENT IN AML BLASTS SUB-POPULATION SORTED BY ITS CD34 EXPRESSION

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Background. Mutations within the FMS-like tyrosine kinase 3 (FLT3) gene have been detected in 20-25% of acute myeloid leukemia (AML) patients, corresponding with poor prognosis. Intact FLT3 is associated with normal hematopoiesis and is over-expressed in CD34+ leukemic blasts. Internal tandem duplication (ITD) mutation increases FLT3 kinase activity and may play a role in leukemogenesis. Leukemia stem cells reside mainly in the CD34+ compartment. Therefore, interest is growing in comparing FLT3 status in specific CD34-/+ blasts sub-populations. **Aims.** To examine the presence of FLT3-ITD mutation in both CD34+/- blasts sub-populations of adults AML patients known to carry the mutation. **Methods.** Six AML patients diagnosed with FLT3-ITD mutation were evaluated. AML blasts were sorted by FACSAria flow cytometer into CD34+ and CD34- groups. DNA was extracted from each cell population and the presence of FLT3-ITD mutation was detected following PCR reaction (amplifying exons 11 and 12). **Results.** High quality DNA was extracted from blasts sub-populations in five patients. Flow cytometry analysis validated that both CD34+ and CD34- sorted cells express abnormal (leukemic) phenotype. In two patients, FLT3-ITD mutations were evident in both compartments (Figure 1. patients A and B). In the other three, FLT3-ITD mutation was selectively expressed in only one sub-population as shown in Figure 1 (patients C and D; patient E result is not shown). Patient C is a 37 year old male. On diagnosis 95% of his blasts were CD34 positive. Sorting revealed that the FLT3-ITD mutation was specific to the CD34 positive cells. Patient D is a 34 years old male. On diagnosis he presented with NPM1 positive, FLT3-ITD negative mutation and a CD34 negative phenotype. Unfortunately, he relapsed two months after concluding his treatment plan. On relapse, the NPM1 mutation was not detected and two distinctive CD34+/- blast populations were observed. A new FLT3-ITD mutation was detected solely in the CD34- sub-population. Patient E is a 67 year old male. On diagnosis 40% of his blasts were CD34 positive. His FLT3-ITD mutation signal was weak, corresponded with a low burden of mutated allele, but was apparent only in the CD34+ compartment. **Conclusions.** FLT3-ITD mutation is not considered the leukemia initiating event, and therefore occurs later in leukemogenesis. It is known as a poor prognostic factor but yet, the underlying mechanism is not clear. The ability to separate blast populations harboring FLT3-ITD mutation and compare them to blasts from the same patient expressing wt-FLT3 may help to understand FLT3-ITD mutation biological effect. In three out of five examined patients FLT3-ITD mutation reside only in blasts sub-population differentiated by CD34 expression. Our results indicate that leukemic blasts may be divided in some cases into distinct phenotypic and genetic properties populations. This may serve as an opportunity to better explore late events during leukemogenesis.

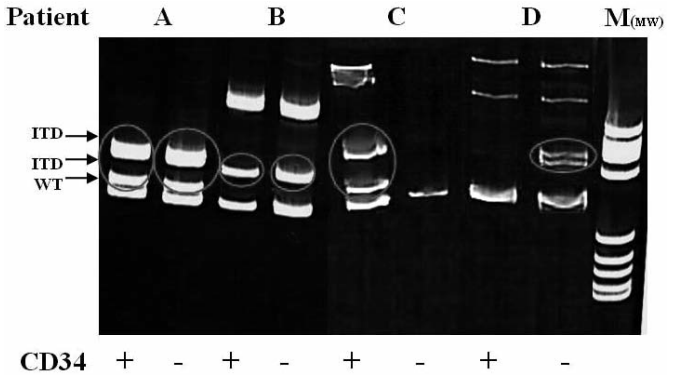

Figure. 1. PCR analysis of FLT-3-ITD mutation. Acrylamide gel electrophoresis of PCR products with (red circle) and without FLT3-ITDs.

1201

DEVELOPMENT AND APPLICATION OF AN ASSAY FOR IDENTIFICATION OF PROGNOSTIC MUTATIONS IN AML PATIENTS

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Background. In acute myeloid leukemia (AML) with a normal karyotype, which constitutes approximately 50 % of all AMLs, the genetic basis for the disease seems to arise from somatic mutations. During the past years, numerous somatic mutations with prognostic implication have been identified and described for this subgroup of patients. **Aims.** To develop a convenient method for identification of prognostic mutations in the genes *FLT3 (ITD* and *D835*), *c-KIT (D816V)*, *IDH1* (*R132*), *CEBPA*, *NPM1*, and *WT1* (*exon 7*) and to apply the assay to a cohort of 73 AML patients diagnosed at our department. **Methods.** Fragment analysis using fluorescence labeled PCR products was developed for detection of indel mutations in *FLT (ITD)*, *CEBPA*, and *WT1* (*exon 7*) while restriction endonuclease-mediated selective PCR (REMS-PCR) in combination with fragment analysis was designed for detection of the point mutations *FLT3-D835*, *c-KIT-D816V*, and *IDH1-R132*. **Results.** In a pilot study of a cohort of 73 AML patients, we found one or more mutations in 45. 2% of all patients (one mutation: 26. 0 %; two mutations: 15. 1 %; three mutations: 4. 1 %). The distribution and frequency of mutations differed between patients in age groups <60 years and >60 years. The *IDH1-R132* and *FLT3-D835* mutations were not found in the patients < 60 years while it constituted 41. 9 % of all mutations in patients > 60 years. Furthermore the WT-1 mutation was found with a higher frequency in the younger group of patients. *Discussion:* This newly developed assay identified prognostic mutations in a high proportion of patients with AML. The assay is easily applicable in a clinical routine setting and enables upfront prognostication of the individual patient. In addition, the achievement of molecular phenotypes in the heterogeneous group of AML patients, not defined by chromosomal translocations, enable the clinician to follow minimal residual disease in more patients.

1202

P45NF-E2 FUNCTIONS AS TUMOR SUPPRESSOR GENE IN F-MULV INDUCED ERYTHROLEUKEMIA BY INDUCTION OF G1 CELL CYCLE ARREST

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Background. Nuclear factor erythroid 2 (NF-E2) is one of the most important transcriptional factor to erythroid differentiation, its p45 subunit is critical for the normal globin and platelet production. Frequent proviral insertional inactivation of the p45NFE2 locus in Friend murine erythroleukemia virus (F-MuLV) induced erythroleukemia cells, combined with the evidence that p45NFE2 mutant mice succumb to the disease significantly faster than control mice and that the absence of p45NFE2 in erythroleukemic cells promotes tumor growth by accelerating the rate of cellular proliferation, has defined a pivotal role for NF-E2 in the progression of F-MuLV induced erythroleukemia. **Aims.** The aim of this study was to explore the mechanism of the anti-tumor effect of p45NF-E2 by reintroduced the gene into non-producing erythroleukemia cells produced by F-MuLV integrational inactivation. **Methods.** p45NF-E2 gene was transfected into non-producing erythroleukemia cell line HB22. 2 with constructed pEF-NFE2 plasmid and the expression of α-globin was detected with Northern blot to determine the gene activity. The cell proliferation was assayed by depicting the growth curve and the cell cycle was detected by flow cytometry. The expression of cell cycle relative protein was detected by Western blot. And the interaction between proteins was detected by Co-immunoprecipitation. **Results.** The reintroduction of p45NFE2 in HB22. 2 cells attenuated the growth of erythroleukemic cells in culture through G1 cell cycle arrest that associated with the marked increase of the expression of p27 Kip1 and decrease of CDK2. The interaction between p27 and CDK2 was decreased. Up-regulation of p27Kip1was regulated post-translationally, partially by reduced degradation by phospho-ERK which was shown marked reduction in the cells reintroduced with p45NFE2 compared to the control cells. **Conclusions.** By this study, initial evidence was provided that the erythroid transcription factor, p45NF-E2 can function as tumor suppressor by attenuating the growth rate of F-MuLV-induced erythroleukemia cells and inactivation of its expression contributes to the progression of F-MuLV-induced erythroleukemia.

1203

IMMUNOPHENOTYPIC AND MOLECULAR FEATURES OF 'CUPLIKE' AML

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Background. Nuclear invaginations, also referred to as fishmouth or cuplike nuclei, are often identified in microgranular APL, myelomonocytic and monocytic AMLs. More recently, this typical morphological feature has been associated with NPM1 and Flt3 mutations, as well as with the lack of CD34 or HLA-DR expression, in non APL and non myelomonocytic or monocytic AMLs. **Aims.** In this study we retrospectively analyzed the morphologic, immunophenotypic, cytogenetic and molecular features of 68 AML patients admitted to our Institute since

January 2010. **Methods**. A cuplike morphology was defined by the presence of >10% of blasts showing nuclear incision spanning >25% of the nuclear diameter at May Grunwald-Giemsa staining, independently assessed by two hematologists. Four-color or 6-color flow cytometry immunophenotypic analysis was performed on bone marrow aspirate using a FACSCanto II with a large panel of monoclonal antibodies; blasts were gated for CD45 expression and light side scatter characteristics. PCR was used to screen for Flt3-ITDs. NPM mutations were detected by immunohistochemical analysis of abnormal localizations in the NPM protein cytoplasm. Conventional cytogenetics was performed on bone marrow aspirate using standard Giemsa trypsin G-banding procedures. **Results**. Sixty-eight patients were included in the study, 39 males and 29 females, with a median age of 64 years (26-84). According to FAB criteria, 9 cases were classified as M0, 15 as M1 and 44 as M2. A cuplike nuclear invagination was detected in 15 (22%) cases; the cuplike blasts showed a relatively scanty, pale basophilic cytoplasm with little granularity; there was a 38% median percentage of blast cells with nuclear invagination (range: 10-80). No difference was observed between cuplike and control cases as regards age, sex, FAB group, WBC and platelet counts, haemoglobin level, percentage of blasts and normal karyotype. Loss of CD34 expression was observed in 53% of cuplike cases vs. only 23% in the control group (p: 0. 03); the same significant difference was observed for the lack of HLA-DR expression (43% vs. 13%, p: 0. 03). In cases with a cuplike morphology, a higher incidence of internal tandem duplication of the Flt3 gene and abnormal cytoplasmic localization of NPM protein were observed (73% and 50% vs. 31% and 24%) but the difference was statistically significant only for Flt3-ITD (p: 0. 02). **Concluions**. Our data show that non promyelocytic-non monocytic AMLs with cuplike nuclear invagination are associated with Flt3-ITD positivity as well as loss of CD34 and HLA-DR expression. This relationship is not sufficient to suggest that they could represent a distinct AML subtype, but further molecular investigations could yield a better characterization of this AML subgroups.

Table 1.

	N	M/F	Lack of CD34	Lack of HLA-DR	FLT3-ITD+	NPM+	Normal Karyotype
Cuplike+	15	7/8	53%	43%	73%	50%	64%
Cuplike-	53	32/31	23%	13%	31%	24%	50%
p			0.03	0.03	0.02	ns	ns

1204

BCL2 ASSOCIATED ATHANOGENE-1 (BAG-1) IS OVEREXPRESSED IN CHILDHOOD AML

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Background. BCL2 associated AthanoGene-1 (BAG-1) represents a pro-survival protein. In humans, it can be found as three, diverse in length, protein isoforms (BAG-1L; BAG-1M; BAG-1), characterized by BAG-domain, and Ubiquitin-like domain which mediates BAG-1 / proteasome interaction. Recently, we showed that BAG-1 was highly expressed in leukemic cell lines. We demonstrated that BAG-1 exerted a pro-survival role by disabling proteasome dependent degradation of anti-apoptotic protein BCL2. The same was observed for USP9X deubiquitinase, which was revealed to be a key BAG-1's target. In fact, USP9X kept the anti-apoptotic MCL1 protein away from degradation in our model. As a result, leukemic cells were well protected from apoptosis induction, and hence, had better surviving capacity. **Aims.** We aimed to confirm our in vitro studies about BAG-1, BCL2 and MCL1 in pediatric patients affected by acute leukemia. **Methods.** RQ-PCR and Western Blot (WB) study is performed to study BAG-1's expression in 10 patients affected by acute myeloid (AML) and lymphoid leukemia (ALL). Reverse-phase protein array (RPPA) analysis was conducted in large cohort of AML (n=66) patients at diagnosis. **Results.** The mRNA expression of BAG-1 showed to be heterogeneous in acute leukemia at diagnosis being not so informative. Its protein expression was higher with respect to healthy bone marrow (hbm) where it was undetectable by WB. We found the tendency of mitigating protein expression of either all three of BAG-1 isoforms in ALL, or in particular of BAG-1 in AML samples during remission phase suggesting a putative BAG-1's role in leukemia onset. By RPPA, we showed that BAG-1 protein was overexpressed in 56 AML patients (84. 8 %) with respect to hbm (mean AML intensity value (m. i. v): 113616 ± 66721; fold increase: 2. 36). We then studied the expression of direct or indirect BAG-1 interacting partners previously identified. We confirmed a significant ($p < 0.001$) correlation between BAG-1 elevated levels and high BCL2 protein levels (m. i. v = 110033 ± 89117), high BCL2S70 (m. i. v: 70387 ± 40864; $p < 0.001$), and high MCL1 (112309 ± 49254; $p < 0.001$). Then, we considered patients for the typical clinical biological features (karyotype, molecular genetics, FAB, age and WBC). We highlighted a relationship with the highest BAG-1 expression and normal karyotype group of patients, who are known for a poor prognosis. Whereas, the lowest BAG-1 protein expression, found in 15. 2 % of analyzed patients, is connected with t(8;21) AML-ETO translocation (found in 9/10 patients with low BAG-1 expression; $p < 0.001$), who are stratified in standard risk group and have a better prognosis. **Conclusions.** Our results revealed the tendencies of pediatric AML to express highly BAG-1 at diagnosis, as well as BCL2 and MCL1. We supported BAG-1 protein to be unraveled as a novel marker in AML, and to be considered for future therapeutical opportunities.

1205

THE EFFECT OF PROLIFERATION INHIBITION AND APOPTPSIS INDUCTION OF ICARIIN ON NB4 LEUKEMIA CELLS AND ITS MOLECULAR MECHANISMS

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Background. Acute promyelocytic leukemia (APL) is a special type of acute myeloid leukemia, and bleeding symptoms are very prominent. Since the usage of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), prognosis of APL has been significantly improved. However, there are still 40% patients relapse after treatment with ATRA and ATO, and ATRA results in serious Retinoids Acid Syndrome in some cases. Hence, new regimens are necessary for patients of APL. Icariin (ICA) is traditional Chinese drug which is derived from several species of plants in the Epimedium family, commonly known as Horny Goat Weed or Yin Yang Huo. It has been reported that ICA can inhibit the proliferation of K562 and HL-60 cell lines, and induces apoptosis of HL-60 cell line. However, little is known about the effect of ICA on APL cell line. **Aims.** To investigate effects of ICA on proliferation, cell cycle and apoptosis of NB4 cells and its underlying anti-tumour mechanisms. **Methods.** The inhibitory effect of ICA on proliferation of NB4 cells was assayed by MTT. Cell cycle and apoptosis of NB4 cells treated with ICA were detected by flow cytometry (FCM), Western blotting was employed to access the role of BAX and BCL-2 family proteins in the apoptosis pathway of NB4 cells. . **Results.** ICA could produce a significant cytotoxicity on NB4 cells in a dose-dependent (0, 4, 8, 16, 32 and 64 μmol/L) and a time-dependent (24, 48 and 72 hours) manners, for 48hours, inhibition rate of NB4 cells increased from 22. 80%±0. 65% to 74. 29%±0. 89%($F=21.493$; $P<0.01$), ICA inhibited NB4 cells growth with IC50 of 18. 48 μmol/L. Compared with the vehicle-treated controls, the NB4 cells treated with ICA at a concentration of 8, 16, 32 and 64 μmol/L for24 hours, were arrestted in G1 phase. Meanwhile, ICA could induce a significant apoptosis effect at all the series of concentrations. After incubated with 4, 8, 16 and 32 μmol/L ICA for 24 hours, apoptosis rate of NB4 cells increased from 9. 73% ± 0. 85% to 28. 45% ± 0. 99% ($F=16.183$; P□0. 05). Further research found ICA treatment of NB4 cell resulted in a decrease the levels of antiapoptosis Bcl-2 protein, whereas a concomitant increase proapoptosis Bax protein, and the effect was in a dose-dependent manner. The Bax/Bcl-2 ratio of NB4 cells was significantly increased in a dose-dependent manner treated with ICA. **Conclusions.** The present study suggested that ICA could significantly inhibit the proliferation and promote apoptosis of NB4 cells in a s dose and time-dependent manner. Furthermore, the result also demonstrated that ICA could arrest cell cycle in G1 phase, and significantly down-regulate BCL-2 and up-regulate Bax proteins, by which the apoptosis of NB4 cells was initiated. Key words Icariin; NB4 cells; Acute promyelocytic leukemia; Proliferation;Apoptosis; Bax; Bcl-2.

1206

RISK STRATIFICATION OF ACUTE MYELOID LEUKEMIA: A SICILIAN NETWORK FOR INTEGRATIVE ANALYSIS OF MULTIPLE MOLECULAR MARKERS AND KARYOTYPE

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Background. Acute myeloid leukaemia is a cytogenetically heterogeneous disorder with acquired recurrent chromosomal alterations detected in about 55% of patients. The remaining cases, 40-50% of AMLs, have normal karyotype and are characterized by an heterogeneous group of molecular abnormalities. In normal karyotype AML (NK-AML), molecular alterations with adverse prognostic impact include gene mutations of FLT3, WT1, IDH1, DNMT3A and high expression levels of the BAALC, ERG and MN1 genes, whereas favourable prognosis is associated with the presence of mutations in the CEBPA and NPM1 genes. **Aims.** We planned to perform a regional study to realize a bio-bank of molecular characterized leukemic blasts. All the Haematology Sicilian Centre are involved and the objectives are to valuated the incidence of the molecular aberration in our region and to offer to the clinicians useful information about the biologic risk-category of patients. Preliminary results on 147 consecutive AML patients enrolled in the study, during the year 2011, are reported. **Methods.** Nucleic acids and leukemic cells at diagnosis were collected and stored according to standard protocols. Cytogenetic studies were performed according to standard protocols. Molecular characterization included fusion gene (PML/RARalfa, BCR/ABL, AML1/ETO, CBFB/MYH11, MLL rearrangements); gene mutations (FLT3, NPM1, WT1, IDH1, IDH2 and DNMT3A); gene expression (WT1, BAALC and MN1). AML1/eto and CBFB/MYH11 (CBF leukaemias) were also characterized for KIT gene mutation. **Results.** We enrolled 147 AML cases from across the region: 68 cases from western sicily and 79 cases from eastern sicily . We identified 10 APL carrying PML/RARAalfa fusion gene, the remaining 137 AML cases were so distributed: 26 AML NPM1 mutated (19%), 31 FLT3 mut (23%) (29 with FLT3 ITD and 2 with D835), 2 AML cases showed WT1 mutations, 6 AML cases showed R132C IDH1 mutations, 4 AML cases showed R172 IDH2 mutations, 6 CBF AML cases 1 of them showing D816V KIT mutation, no one AML showed BCR/ABL fusion gene, 1 AML case showed MLL rearrangements, 6 AML cases showed R882H DNMT3A mutations. Cytogenetic studies available on 94 cases allow to identified 59 normal karyotype, 14 adverse, 14 intermediate karyotype, 7 favourable karyotype. Gene expression analysis of WT1 showed a median of 7304 copies/104 *ABL* copies (range from 2 to 26x10E6). 50 NK-AML patients were divided into quartiles by gene expression levels of BAALC and MN1 and into low and high expressers using the median expression level. Patients were scored as high risk when showed high expression of both genes and as low risk when showed low expression values of both genes. . Integrative analysis of a large molecular panel of markers and cytogenetic characteristics allow us to perform a prognostic stratification in high and low risk at disease onset in more than 80% of patients, only 10-20% of patients remained in the less informative "intermediate"category. **Conclusions.** Assessment of a exhaustive molecular and cytogenetic study at the presentation of the acute leukaemia may be very helpful to appropriately tailor the aggressiveness of therapy in the great majority of AML patients.

1207

HYPERDIPLOID KARYOTYPE IN ACUTE MYELOID LEUKEMIA WITH MDS-RELATED CHANGES PATIENTS

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Background. Acute myeloid leukemia with myelodysplasia-related changes is an acute leukemia presenting usually complex karyotype, various lineage myelodysplasia and generally poor prognosis. The group of AML with complex karyotypes is heterogenous and may include monosomal as well as hyperdiploid karyotypes. In hematological neoplasms several cytogenetic findings are associated with morphologic abnormalities. Except for usual trisomy 8 and rare gains of chromosomes 9,11 or 21, hyperdiploidy defined as >46 chromosomes has rarely been reported in MDS and AML. **Aims.** the aim of this study was to characterize the ploidy status of karyotype in accordance with bone marrow dysplasia. **Methods.** Over the last two years, 58 patients were referred to our institution for evaluation and treatment of *de novo* AML. These patients did not present with recurrent cytogenetic abnormalities. Conventional cytogenetic analysis was performed on G-banded metaphase cells prepared from bone marrow aspirate cultures using standard techniques. Bone marrow aspirate smears were assessed according to criteria for dysplasia stated in the 2008 WHO classification. **Results.** Twenty three patients under 60 years of age (22. 4%) were diagnosed as an AML with MDS-related changes according established criteria and 13 of this group had a complex karyotype with ploidy changes. According to combined FAB- immunologic classification the patients were diagnosed as: AML M1-5 pts, M2-1 pt, M4-4 pts, M5-2 pts, M6-1 pt. None of molecular markers including EVI1 by Q-PCR, FLT3-ITD by PCR and MLL-PTD by RT-PCR methods were detected. Based on the ploidy status, cases were stratified into two groups: presenting monosomal karyotype (7 pts, 53. 8%) and high hyperdiploid karyotype (6 pts, 46. 1%) with chromosomes number ranged from 49 to 96 Recurrent karyotype abnormalities presented gains of chromosomes 1, 8, 11, 13,15, 21, 20, 21, 22 and only one patient had structural changes: add(19)(q13),+mar. The most frequent dysplastic features of bone marrow in presenting monosomal karyotype patients group were megaloblastic changes and multinuclearity in erythroblasts, hypogranulation, hyposegmentation and gigantism in granulopoesis and presence giant forms of platelets and micromegakaryocytes in the megakaryocytic lineage. In all patients in the group with high hyperdiploid karyotypes marked dyserythropoesis characterized by presence of multinucleated cells especially poli-and ortochromatofil erythroblasts was observed. The overall outcome was extremely poor with early death in almost all patients. **Conclusions.** This finding has suggested that DNA content per se affects cellular functions and seems to be another poor prognostic factor in the AML patient group. This particular hyperdiploid pattern of karyotype remains associated with marked dyserythropoiesis in bone marrow and should be considered as a unique group with extremely poor prognosis.

1208

CD200 EXPRESSION IN MYELOID LEUKEMIC BLASTS

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Background. CD200 is a membrane glycoprotein, belonging to the immunoglobulin superfamily, expressed on a subset of T and B lymphocytes, endothelial cells, dendritic cells and neurons. The overexpression of CD200 has been implicated in the pathogenesis of solid tumors and hematological malignancies including lymphoma, myeloma, chronic lymphocytic leukemia and acute myeloid leukemia (AML). Previous studies have shown that expression of CD200 on AML blasts is associated with a poor prognosis. **Aims.** Assess the frequency of expression of CD200 in myeloid leukemic blasts and the correlation with the expression of other antigens. **Methods.** The study included 24 patients, 13 male and 11 female, with a median age of 73 years (range 52-94); of these 20 with diagnosis of AML (5 M0, 1 M1, 5 M2, 2 M3, 5 M4, 2 M5 according to FAB criteria) and 4 with diagnosis of refractory anemia with excess blasts-2 (RAEB-2). Flow cytometry immunophenotype of bone marrow and peripheral blood were analyzed using a panel of monoclonal antibodies included CD200, CD13, CD33, CD34, CD14, CD15, CD2, CD3, CD7, CD19, CD20, CD56, CD117, HLA-DR. **Results.** CD200 was present on blast cells of 13/24 patients (54. 1%). In all 13 patients CD200 was co-expressed with CD34, CD117 and HLA-DR. CD13 and CD33 were co-expressed in 12/13 and 8/13 patients respectively. No patient co-expressed CD200 and CD14, CD15, B and T lymphoid antigens. Only one patient co-expressed CD200 and CD56 and one CD200 and CD7. On contrary of the 11 patients that did not express CD200 only 5 expressed CD34, while CD117 was expressed in 10/11 and HLA-DR in 9/11. CD13 and CD33 were expressed in 10/11 patients while CD14 and CD56 in 2/11 and 3/11 respectively. The expression of CD200 in the FAB subgroups was 5/5 in M0 (100%), 1/1 in M1 (100%), 2/5 in M2 (40%), 0/2 in M3, 4/5 in M4 (80%), 0/2 in M5. As regards the four RAEB-2, two expressed CD200. **Conclusions.** In our cohort of patients, the expression of CD200 was 54. 1%. In particular the expression of CD200 is associated with CD34+ AML blasts in all cases, while is not expressed on more differentiated myeloid cells (0/13 co-expressed with CD15) or on monocytes (0/13 co-expressed with CD14); moreover CD200 was not expressed in the two M3 and in the two M5 and was expressed only in 40% of M2 AML. In our series CD200 can be considered as a marker of myeloid blast cells less differentiated and introduced into the panel of immunophenotypic characterization of AML. Further studies are need to investigate the prognostic significance of CD200 expression in myeloid leukemic cells.

1209

HIGH EXPRESSION OF MIR-181A PREDICTS LONGER SURVIVAL OF PATIENTS WITH ACUTE LEUKEMIA - PRELIMINARY DATA

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Background. MicroRNAs (miRNAs) are small non-coding RNA molecules, that control gene expression by targeting messenger RNA (mRNA), via degradation or suppression of translation. miRNAs play important role in many cellular processes, including cell growth and proliferation, differentiation, metabolism and apoptosis. **Aims.** The aim of the study was to determine expression of miR-181a in acute leukemia patients and its influence on patient clinical outcome. **Methods.** miRNAs from isolated leukemic cells were extracted using mirVana™ miRNA Isolation kit (Ambion Inc., Carlsbad, CA, USA) following the manufacturer's protocol. Reverse transcriptase (RT) reactions were performed

for mature miRNA cDNA synthesis in separate tubes using specific stem-loop RT primers and TaqMan® MicroRNA™ Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). After microRNA isolation, reverse transcriptase reactions were performed, followed by cDNA amplification. The relative amount of microRNA-181a was normalized according to the reference RNU48 level. Results were considered statistically significant with p-value < 0. 05. **Results.** 30 patients (11 women and 19 men), median age 58 years (range 32-86) were included into the study. There were 26 patients with acute myeloid leukemia (FAB classification: M0 - 1, M1 - 7, M2 - 9, M3 - 1, M4 - 7, M5b - 1) and 4 patients with acute B-lymphoblastic leukemia. 9 patients had intermediate cytogenetic risk and 21 patients had high cytogenetic risk. The patient bone marrow samples were obtained pretreatment. Median expression of miR-181a was 0. 9203337 (range 0. 017784-224. 1603, SD 43. 9080). Higher expression of miR181a was positively correlated with lactate dehydrogenase level LDH (p=0. 038) and negatively correlated with anemia (p=0. 01). There was no correlation between expression of miR-181 and cytogenetic risk group, achieving complete remission and risk for disease relapse. Higher expression of miR-181a was positively correlated with longer survival of acute leukemia patients (p=0. 02). **Conclusions.** We proved that higher expression of miR-181a positively correlated with acute leukemia patients overall survival. As the study includes a small number of patients it needs confirmation on a bigger cohort.

1210

VEGF MRNA LEVELS, PROTEIN CONCENTRATION AND MICROVESSEL DENSITY IN AML PATIENTS, BEFORE AND AFTER TREATMENT IN NORTH-WEST OF IRAN

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Background. Angiogenesis, the formation of new blood vessels from pre-existing ones, plays a crucial role in the pathophysiology of solid tumors and hematologic malignancies. In AML, the increased micro vessel density (MVD) is the result of the action of several angiogenic growth factors secreted by leukemic blasts. Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), transforming growth factor (TGF) and the angiopoietins (Ang) are recognized as angiogenic growth factors. Vascular endothelial growth factor plays an essential role in normal and pathologic angiogenesis but its clinical role in AML is unclear. **Aims.** As the prognosis of AML patients may be correlate with the degree of angiogenesis and VEGF, we investigated the expression of VEGF-C and VEGF-A, evaluate MVD in the bone marrow, levels of VEGF-C in the bloodstream of patients with newly diagnosed AML before and after treatment. **Methods.** A Interventional before and after study of 31 patients with newly diagnosed AML was conducted in Hematology-Oncology Research Center in Tabriz -Iran from 2009 to 2010. We investigated the mRNA expression of VEGF-A and VEGF-C from bone marrow of thirty-one patients with newly diagnosed AML before and after treatment. Real- time Quantitative RT-PCR assay was performed in order to detect the mRNA levels of VEGF. Bone marrow biopsy from all AML patients were assay for micro vessel density (MVD), using anti-CD 34 monoclonal antibody. Serum concentrations of VEGF in 31 AML patients were measured by ELISA assay. Allpatients, recovered combination chemotherapy with cytosine arabinoside[Ara-C] and an anthracyclin. **Results.** 20 (62%) patients were male while 11(38%) were female. The mean age was 36+1. 4. The mean amount of MVD was reduced from 10. 8±3. 6 to 7. 6±3. 3 after treatment (P=0. 008). VEGF levels were also reduced from 0. 59±0. 16 to 0. 24±0. 03 after treatment (P=0. 005). Gene expression differences for VEGF-A, mRNA was 4. 6±1. 4 while it was 120. 7±93. 2 for VEGF-C mRNA, which was significant only for VEGF- A mRNA (P=0. 02). **Summary and Conclusions.** According to these results, AML is associated with an increased expression of VEGF-A and VEGF-C mRNA after therapy. It seems that VEGF had a tumor inhibitory role and up-regulation of VEGF-A and VEGF-C inhibits the growth and progression of AML leukemic cells. We can conclude that anti-angiogenesis preparations can be effective in treatment course of AML in combination with chemotherapy regimen.

1211

ASPARAGINE SYNTHETASE AS A BIOMARKER OF ASPARAGINE DEPLETION EFFICACY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Asparagine synthetase (ASNS) is an enzyme converting aspartate to asparagine, which is the target of L-asparaginase. Thus, its low expression in tumor cells is susceptible to represent a marker of sensitivity to L-asparaginase. In clinics, several cases of successful asparaginase treatment in monotherapy have been reported in patients with acute myeloid leukemia (LAM). Capizzi *et al*. also demonstrated that, in adults patients with refractory or relapsing LAM, sequential administration of cytarabine and asparaginase (CLASP) was more efficient than cytarabine alone (40% vs. 24% of complete remission in refractory patients, p=0. 02). Moreover, several recent *in vitro* studies suggest that the efficacy of asparaginase to treat LAM is inversely correlated with ASNS expression in leukemic cells. Asparaginase encapsulated into red cells (GR-aspa) shows a tolerance profile that allows considering a secure treatment of LAM by asparaginase, particularly in elderly patients. **Aims.**To evaluate the *in vitro* sensitivity to asparagine depletion of myeloid tumor cells from AML patients and to determine if the expression of ASNS is correlated to this sensitivity. **Methods.** Total RNA will be immediately extracted (PAXgene® kit) from bone marrow cells of AML patients and ASNS mRNA expression will be evaluated by qRT-PCR. Blasts will be isolated by Ficoll separation and ASNS protein expression will be assessed by western-blot. Sensitivity to asparaginase of the isolated blasts will be evaluated *in vitro* by incubating the cells for 4 days with concentrations of asparaginase ranging from 0. 01 to 10 IU/mL and by measuring the cytotoxicity (IC50) with a cell counting CCK-8 kit. **Results.** ASNS protein and mRNA expressions of the leukemic cells will be compared to the IC50 for each patient to determine if a correlation is observed between those parameters. Our preliminary results obtained *in vitro* with lymphoid and myeloid cell lines (MOLT-4, HL-60) clearly show a correlation between a low expression of ASNS and a good sensitivity to asparaginase. In addition, these results will be compared to FAB/WHO classifications of LAM to evaluate the possible impact on asparaginase sensitivity. **Conclusions.** Selection of AML patients based on their level of ASNS expression may be necessary in further clinical trials evaluating the efficacy of asparaginase encapsulated into red blood cells.

1212

IDH1 MUTATIONS ASSOCIATES WITH UNFAVOURABLE PROGNOSIS IN YOUNGER ADULTS WITH NPM1 MUTATED ACUTE MYELOID LEUKAEMIA.

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Background. During the last decade it has become clear that gene mutations add important prognosis information to the cytogenetic subtypes in acute myeloid leukemia (AML), mostly in patients with normal karyotype (NK). More recently, a novel mutation affecting the arginine residue at position 132 of the *IDH1* gene (NADPH dependent isocitrate dehydrogenase 1), an enzyme from the citric acid cycle, has been described in AML patients. Different studies suggested an association with NK and a probable unfavourable impact in AML in terms of progression free survival (PFS) and overall survival (OS). **Aims.** To analyze the influence of IDH1 mutations in NK-AML, particularly in patients without FLT3-ITD, classified as intermediate risk (IR) o low risk (LR) who could benefit with more intensive treatment. **Patients and Methods.** The study comprised 23 NK-AML patients diagnosed in our hospital during the last 5 years (2007-2011). Clinical characteristics, treatment administrated, FLT3/NPM1 mutations and outcomes are shown in Table 1.

Table 1.

Pat	Age	FAB/WHO	FLT3-ITD	NPM1	IDH R132	Risk	BM Post-Ind	BM Post-Cons	SCT*	Relapse	PFS	Exitus	Cause of death	LFU
1	60	M1	N	P	G395T	LR	CR, NEG MRD	CR, NEG MRD	AUTO	YES	505	YES	Progression	843
2	38	M4	N	P	G395A	LR	CR, NEG MRD	ND		YES	425	NO		936
3	62	M1	N	P	C394T	LR	CR, NEG MRD	CR, NEG MRD	AUTO	NO		NO		804
4	18	M1	N	P	N	LR	CR, NEG MRD	CR, NEG MRD		YES	435	YES	Progression	613
5	34	M4	N	P	N	LR	CR, MRD POS	CR, EMR POS	DUAL	NO		NO		912
6	62	M4	N	P	N	LR	CR, MRD POS	CR, NEG MRD	AUTO	YES	821	YES	Progression	873
7	54	M4	N	P	N	LR	CR, NEG MRD	CR, NEG MRD		NO		NO		782
8	59	M4	N	P	N	LR	CR, NEG MRD	CR, NEG MRD	AUTO	NO		NO		662
9	55	M4	N	P	N	LR	CR, NEG MRD	CR, NEG MRD		NO		NO		315
11	56	M1	N	P	N	LR	PR,MRD POS	PR, POS MRD		NO		NO		219
12	51	M2	N	N	N	IR	CR, NEG MRD	CR, EMR POS	DUAL	NO		NO		253
13	44	M4	N	N	N	IR	CR, MRD POS	CR, NEG MRD	ALO	NO		NO		434
14	42	M4	N	N	N	IR	CR, MRD POS	CR, EMR POS	ALO	NO		NO		845
15	51	M6	N	N	N	IR	CR, NEG MRD	CR, NEG MRD	ALO	NO		NO		778
16	44	M1	N	N	N	IR	CR, NEG MRD	CR, NEG MRD	ALO	NO		NO		1144
17	40	MD-AML	N	N	C394T	IR	CR, MRD POS	CR,NEG MRD		YES	190	NO		235
18	42	M2	P	P	G395T	HR	CR, NEG MRD	CR, NEG MRD	ALO	NO		NO		1357
19	38	M2	P	P	N	HR	CR, NEG MRD	CR, NEG MRD		YES	160	YES		240
20	51	M4	P	P	N	HR	CR, NEG MRD	ND	MUD ALO	NO		YES	Pneumonitis	728
21	51	M5b	P	P	N	HR	CR, MRD POS	CR, EMR POS	ALO	NO		YES	Sepsis	195
22	32	M5a	P	P	N	HR	CR, NEG MRD	CR, NEG MRD	DUAL	NO		NO		350
23	37	M2	P	P	N	HR	CR, MRD POS	CR,NEG MRD	MUD ALO	NO		NO		202
24	41	M2	P	N	N	HR	CR, MRD POS	CR, EMR POS	MUD ALO	NO		NO		364

N, negative; P, positive; L, low; I, intermediate; H, high; Ttreatment. induction IA(3x7) all cases except ICE in #16; Consolidation IA(3x7) all cases except IA(2x5) en #19. *Only SCT performed before relapse are considered. LFU: Last follow up (days from diagnosis)

Genomic DNA was purified, after written informed consent, from bone marrow (BM) samples at diagnosis. IDH1 mutations were determined by direct DNA sequencing as previously reported (Balss et al, Acta Neuropathol 116, 2008).

Results. The frequency of IDH1 mutation in the present series was in accordance with previous reports, being 21,8% (5/23) including 30% (3/10) FLT3neg/NPM1pos, 16,7% (1/6) FLT3neg/NPM1neg and 14,3% (1/7) FLT3pos patients (Table 1). Within the 10 LR patients according to FLT3/NPM1 mutations (neg/pos), 2/3 (67%) IDH1pos patients relapsed while only 2/7 (28,5%) IDH1neg did. Interestingly, one of these two relapsing IDHneg patients showed WT1 gene mutations, which has also been associated with poor prognosis. Moreover, from the 6 patients with IR according FLT3/NPM1 mutations (neg/neg), only one showed IDH1 mutations and progressed during treatment before stem cell transplantation (SCT; Table 1). **Conclusions.** IDH mutations are frequent in NK-AML patients, particularly in those with NPM1 mutation. IDH1 mutations are associated with an increased incidence of relapse in the subset of NK-AML with mutated *NPM1* without *FLT3*-ITD (patients LR). According to the IDH mutation status, such patients should not be assigned to the LR group and would probably benefit from more intensive therapies in the first complete remission, including stem cell transplantation

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PROGNOSTIC RELEVANCE OF WILMS' TUMOR 1 (WT1) GENE MUTATION IN-PATIENT WITH ACUTE MYELOID LEUKEMIA

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This study aimed to assess the prognostic impact of Wilms' tumor 1 (WT1) gene mutations in cytogenetically normal acute myeloid leukemia (CN-AML) among Egyptian patients. Exons 7 of WT1 was screened for mutations in samples from 82 CN-AML patients out of 203 newly diagnosed AML patients, age range from 21-74 years, using a high-resolution capillary electrophoresis. Seven patients out of 82 (8. 3%) harbored WT1 mutations. There was no statistically significant difference between the WT1 mutant and wild type as regard age, sex, French-American-British subtypes and the prevalence of success of induction remission therapy (P = 0. 966; 28. 6% vs 29. 3%). Patients with WT1 mutations had overall survival (OS) lower than the wild type one (*HR*= 1. 38; 95% CI, 4. 79 to 6. 86; *P* = 0. 004). *In conclusion,* CN-AML patients with WT1 gene mutation have poor clinical outcome. We recommend testing the WT1 mutations as part of molecularly based risk assessment and risk-adapted treatment stratification of patients with CN-AML.

1214

EXPRESSION OF LEUKEMIA-ASSOCIATED ANTIGEN GENES IN AML PATIENTS AT DIAGNOSIS AND DURING A COURSE OF THE DISEASE

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New molecular markers of residual disease are needed for monitoring of residual disease (MRD) in AML patients. MSLN, ST18, CSPG4 and XAGE1 genes, which belong to leukemia-associated antigen genes (LAA), are possible candidates for this purpose. These genes have high expression in cells of leukemia patients and the background expression found in cells of healthy persons is very low. The level of expression of these genes was estimated by quantitative RT-PCR in leukocytes of AML patients. An overexpression of XAGE1 and CSPG4 genes was found in 29/154 (23 %) patients, CSPG4 in 29/154 (19 %) patients and ST18 in 69/154 (45 %) patients at diagnosis. 8 patients, who neither had mutated NPM1 gene, nor they had overexpressed Wt-1 gene, were monitored by expression of the LAA genes. The results showed a good correlation between the expression of the LAA genes and a clinical course of the disease. Monitoring of MRD by the LAA genes seems to be a suitable marker particularly for AML patients, who do not have any other suitable molecular marker.

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PROGNOSTIC ROLE OF IMMUNOPHENOTYPING IN ACUTE MYELOID LEUKEMIA.

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Immunophenotyping is a major tool to assign leukaemia blast cells to myeloid lineage while the prognostic value of immunophenotyping in Acute Myeloid Leukaemia (AML) is still unclear. The Cytogenetic alterations are the most important prognostic factors in AML and in Myelodisplastic Syndromes (MDS). However it's impossible to have information concerning chromosomal abnormalities from all patients. Therefore we analyzed the pattern of expression of selected antigens: CD13, CD15, CD34, CD33, CD117, DR, CD14, CD64, CD19, in bone marrow blasts of de novo AML by flow cytometry. The aim of the study was to verify the relationship between antigens expression, FAB subtype, karyotype, biomolecular study, and disease outcome (remission rate). We analyzed 72 patients, 51 males and 25 females, median age 58,5 years old (range 15-83) with de novo AML (4 FAB M0, 25 FAB M1, 28 FAB M2, 11 FAB M4 (1FAB M4eo), 7 FAB M5, 1 FAB M6) treated in our department from January 2003 to December 2011 with standard induction chemotherapy for AML. Biomolecular study showed FLT3 mutation in 8 patients and NMP1 mutation in 15 patients. Karyotype was favourable in 2 patients, intermediate in 32 patients and unfavourable in 12 patients. The analysis showed that patients with AML blasts negative for CD13, CD34 and positive for CD33, and CD64 had a higher complete remission rate, independently from FAB subtype risk. This antigen expression was confirmed generally in patients with normal karyotype, age <60 years and normal value of serum LDH . The AML CD13+, CD33+, CD34+, CD117+ blasts were related with poor outcome of disease (partial remission or resistance). HLA DRpositivity appears non specific. Expression of lymphoyd antigen (CD19) was seen in 19 patients. The high expression of CD19 antigen was associated with AML FAB M1 (9 patients) and biomolecular study positive for MLL (1 patient) and complex kariotype. Higher expression of CD13, CD33, CD64, CD14, CD15 was observed in M5b FAB subtype where was obtained complete remission. The low expression of CD34 was associated with NPM mutation and good prognosis. These preliminary data show the prognostic value of immunophenotyping in AML patients and may be useful for risk stratification in this patients. Nevertheless is necessary to analyse a large number of cases for statistical considerations. The AML immunophenotyping should be included as individual prognostic factor.

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NEUTROPENIC ENTEROCOLITIS IN ACUTE MYELOID LEUKEMIA

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Introduction. In this report we focus on the importance of an accurate diagnosis of gastrointestinal complications during chemotherapy for acute myeloid leukemia. The leukemic infiltrtion of the digestive system may cause mucosal ulcers which can lead to bleeding or perforation. The immune system deficiency in this cohort of patients may result in necrotic enterocolitis (leukemic typhlitis), perianal inflammation, abscesses, and peritonitis. **Patients and Results.** Retrospective review of medical documentation of 37 patients with acute leukemia and neutropenic enterocolitis recorded on Clinic of hematology during the four year period (2005-2009). AML M2 was diagnosed in 16 patients, 2 patients had hypoplastic type of AML, 15 patient AML M4, and 4 patient AML M5. All 37 patients with neutropenic enterocolitis received chemotherapy according to protocol MRC AML12 and one received protocol LALA85. 11 patients had unfavorable karyotype. Seven patients had a complex chromosomal abnormalities (more than 3 chromosomal aberrations in more than 2 mitoses), and in four cases monosomal karyotype was registered. At the time of gastrointestinal difficulties all patients were in neutropenic phase (WBC<0. 5x109/l >0. 1x109/l) after receiving induction chemotherapy. For the final diagnosis relevant finding was ileal, cecal or ascedent colon wall tickening larger than 5 mm, verified by utrasonography in all patients. Abnormal findings also included dilated bowel loops (19 patients), air fluid levels (9 patients), dumb - printing (7 patients) and pneumatosis intestinalis (5 patients). All patients were managed with conservative treatment with broad spectrum antibiotic coverage combined with bowel rest, nasogastric suction and total parenteral nutrition. The antibiotic regimens consisted of vancomycin in combination with carbapenem and metronidasole and in combination of cefepime with metronidazole. Twentyfive patients has recovered with just medical treatment after mean time period of 17. 5 days and returned to normal diet. But only 14 of these patients has achieved a complete remission of the acute leukemia. Only two patients were managed surgically. Nine patients died because of neutropenic enterocolitis. Lethal end was the consequence of paralytic ileus (4 patients), mechanical ileus (3 patients) and peritonitis with perforation (2 patient). **Conclusions.** We emphesize the importance of collaboration between the hematologist and the surgeon in monitoring gastrointestinal complications during and after chemotherapy for acute leukemias and diagnostic value of abdominal ultrasonography evaluation.

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IMMUNOPHENOTYPIC PROFILE AND MOLECULAR ANALYSIS OF ACUTE MYELOID LEUKEMIA WITH T(15;17) AND T(8;21)

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Background. French-American-British (FAB) classified acute myeloid leukemia (AML) according to morphology and cytochemistry. FAB-M2 and M3 categories now correspond to World Health Organization (WHO) subcategory of AML with t(8;21) and t(15;17) respectively. WHO in its latest 2008 classification, includes a separate category of AML with balanced translocation/ inversion, which comprises of 7 categories characterized by characteristic genetic abnormalities [i) t(8;21); ii) t(15;17); iii) inv16; iv) t(9;11); v) t(6;9); vi) inv3; vii) t(1;22)] and 2 provisional entities [mutated NPM1 and mutated CEBPA]. It has increasingly been found that, many of these genetically distinct subgroups in AML possess distinctive immunophenotypic profiles, which can be correlated with underlying specific cytogenetic/molecular defects. **Aims.** to evaluate the usefulness of flowcytometric immunophenotyping (FCM-IP) profile in identifying the AML cases with t(8;21) and AML cases with t(15;17). **Methods.** Results of immunophenotypic profile and molecular analysis of AML cases with t(15;17) and t(8;21) were analyzed. In all cases, bone marrow morphologic examination along with cytochemical staining, immunophenotyping and molecular genetic testing was performed. FCM-IP was done using CD1a, CD2, CD3, CD4, CD5, CD7, CD8, cyCD3, TdT, CD34, HLA-DR, CD117, CD56, CD10, CD19, CD22, cyCD22, CD79a, CD13, CD33, MPO, CD11c, CD14 and CD64 on FACS Canto II (BD Biosciences, USA). Molecular testing was done by multiplex RT-PCR (Roche Thermal Cycler, USA). **Results.** From August 2010- December 2011, molecular analysis for t(15;17) and t(8;21) was done in 80 AML cases. Of these, 13 cases were positive for t(15;17) and 17 for t(8;21). Immunophenotyping results were available in 8 cases with t(15;17) and 15 cases with t(8;21). Cases in which immunophenotyping was done were included in this study and analyzed. *AML with t(15;17)*- All cases were strongly positive for MPO cytochemically. Immunophenotyping revealed CD33 and MPO-bright positive; CD34 and HLA-DR- negative; B-lymphoid and T-lymphoid markers- negative in all cases. CD13 and CD117 were also positive in all cases tested, however the intensity varied form dim to moderate. *AML with t(8;21)*- All cases showed moderate to strong positivity for MPO cytochemically. On immunophenotyping, positivity for CD13, CD117, CD33 and MPO were seen in all cases, however, the intensity varied from dim to bright. CD34 and HLA-DR were bright positive in all cases, except 1 where it was dim to moderate. Of the lymphoid markers, CD19 was positive in 6 cases (5- bright positive, 1- dim), CD7 and CD4 in 5 cases each (2- bright positive, 3- dim each) and CD56 bright positive in 2 cases. **Summary and Conclusions.** We found the FCM-IP profile of the two molecularly defined subgroups with t(15;17) and t(8;21), to be clearly distinctive. All cases with t(15;17) were negative for CD34 and HLA-DR, however, these were positive in all cases with t(8;21). Aberrant positivity for lymphoid markers (CD19, CD7, CD4 and CD56) was frequently seen in AML with t(8;21), however, it was not seen in any case with t(15;17). Therefore, cases with a morphologic diagnostic dilemma between FAB-M2 and M3, immunophenotyping can aid in differentiating between the two and also guide towards the underlying genetic defect.

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STUDY ON THE EXPRESSION OF TGF-?1 IN HYPERLEUKOCYTIC ACUTE MYELOID LEUKEMIA

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Background. Hyperleukocytic acute myeloid leukemia (AML) which has an extremely high white blood cell count in peripheral blood, is associated with a high early mortality. The mechanisms for Hyperleukocytic AML have not yet been fully elucidated. TGF-β1 is one of the most potent negative regulator of proliferation, its role in the occurrence of Hyperleukocytic AML is still unknown. **Aims.** To investigate the role of TGF-β1 in pathogenesis of Hyperleukocytic AML by detecting the mRNA transcription and protein expression of transforming growth factor-β1 (TGF-β1) in bone marrow cells and serum levels of TGF-β1 in hyperleukocytic and non-hyperleukocytic AML patients. **Methods.** Bone marrow aspirate samples from 17 hyperleukocytic AML, 21 non-hyperleukocytic AML patients, and 17 normal controls were collected. Informed consents were received from all the patients. The mRNA transcriptional levels of TGF-β1 were detected by real-time fluorescence quantitative polymerase chain reaction (RQ-PCR). The protein expression levels of intracellular TGF-β1 were analyzed by flow cytometry. TGF-β1 serum levels were measured by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). **Results.** (1) The transcriptional levels of TGF-β1: The median levels of TGF-β1 mRNA in hyperleukocytic AML, non-hyperleukocytic AML, and normal control group were 0. 0135, 0. 0259, and 0. 0155, respectively, no statistical difference was observed among these three groups (P>0. 05). (2) The protein expression of intracellular TGF-β1: The mean fluorescence intensity of TGF-β1 in hyperleukocytic AML, non-hyperleukocytic AML, and normal control group were 178±79, 160±54, and 98±18, respectively. TGF-β1 expression in both hyperleukocytic AML and non-hyperleukocytic AML group were significantly higher than that in normal control group (P<0. 05). However, there was no significant difference between the hyperleukocytic and non-hyperleukocytic AML group (P>0. 05). The mean fluorescence intensity of TGF-β1 in AML patients was 168±66, which was significantly higher than that of in normal controls (P<0. 05). (3) Serum levels of TGF-β1: The serum levels of TGF-β1 in hyperleukocytic, non-hyperleukocytic AML, and normal control group were 10. 70±6. 43, 8. 20±6. 34, and 27. 97±14. 55ng/ml, respectively. Serum levels of TGF-β1 in both hyperleukocytic and non-hyperleukocytic AML group were significantly lower than that in normal control group (P<0. 05). There was no difference between hyperleukocytic and non-hyperleukocytic AML patients (P>0. 05). **Conclusions.** (1) This study show that there exists a down-regulation of serum TGF-β1 level in hyperleukocytic and non-hyperleukocytic AML patients, suggesting that TGF-β1 secreted by leukemia cells in AML patients is reduced, so the negative regulation function of TGF-β1 on leukemia cells is diminished. (2) The transcriptional levels of TGF-β1 in hyperleukocytic and non-hyperleukocytic AML patients had no differences with normal controls. However, the expression levels of intracellular TGF-β1 in these patients were increased, with the levels of extracellular TGF-β1 reduced. The results indicate that there may exist abnormality in the process of TGF-β1 secreting outside of cells. (3) The intracellular and serum levels of TGF-β1 between hyperleukocytic and non-hyperleukocytic AML patients have no significant differences. The results show that TGF-β1 hasn't an important impact on pathogenesis of hyperleukocytic AML.

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DO AML PATIENTS WITH DNMT3A EXON 23 MUTATIONS BENEFIT FROM IDARUBICIN AS COMPARED TO DAUNORUBICIN? A SINGLE CENTER EXPERIENCE

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Background. Mutations in *DNMT3A* gene, encoding DNA methyltransferase 3A, were recently described in patients with acute myeloid leukemia (Ley TJ *et al*. NEJM 2010). In 60% of cases, mutations occur in the R882 aminoacid of the methyltransferase domain (exon 23). Most studies have suggested that these mutations confer a poor prognosis in the intermediate-risk AML subset, where this molecular abnormality, could help clinician stratify therapeutic strategy. **Aims.** Because we did not find any prognostic impact in our series (LaRochelle *et al*. EHA 2011), we analyzed the impact of treatments in patients with *DNMT3A* mutations. **Methods.** We determined the mutational status of *DNMT3A* exon 23 in 288 patients aged 18 to 65 years, with newly diagnosed AML in Toulouse University Hospital between 2000 and 2009, excluding promyelocytic leukemia. We focused on intermediate-risk AML and analyzed their outcome depending on the chemotherapy they received (*ie*. type of anthracycline at induction: daunorubicin 180 mg/m² or idarubicin 40 mg/m² total dose, associated to continuous cytarabine 200 mg/m²/d day 1-7). **Results.** A *DNMT3A* exon 23 mutation was detected in 39 patients (13. 5%): 23 R882H, 13 R882C, two R882P and one W893S. Mutations significantly correlated with a higher white blood cell count (median 52 *vs*. 14. 6 G/L, p<0. 001) and a monocytic differentiation (FAB M4/M5, p<0. 0001). All *DNMT3A* exon 23 + patients had intermediate-risk cytogenetics (39/194, 20%). They were associated with *NPM1* (p<0. 001) and *FLT3-ITD* mutations (p=0. 027). No difference in outcome between *DNMT3A* exon 23 + and *DNMT3A* exon 23 - patients was found (CR rate, 87% *vs*. 81%, p=0. 48; median DFS, not reached *vs*. 17. 6 months, p=0. 16 and median OS, not reached *vs*. 24. 7 months, p=0. 17), even after stratifying by *NPM1* or *FLT3-ITD* status. However, *DNMT3A* exon 23 + patients had better median DFS (not reached *vs*. 11. 6 months, p=0. 009) and OS (not reached vs. 14. 3 months, p=0. 005), as compared to DNMT3A exon 23 - patients when treated with idarubicin, whereas patients treated with daunorubicin had similar outcome regardless the *DNMT3A* exon 23 status. In multivariate analysis including age, white blood cell count, *FLT3*-ITD/*NPM1* genotype, anthracycline type and allograft, idarubicin treatment was the only prognostic factor significantly associated with longer OS (HR = 0. 27; 95% CI [0. 08-0. 97]; p=0. 046) in *DNMT3A* exon 23 + patients. **Conclusions.** *DNMT3A* exon 23 mutations have no impact on global outcome in this series, but could be a predictive factor

for response to idarubicin. Other studies testing different modalities of anthracycline administration, especially in the setting of randomized controlled trials (idarubicin *vs*. daunorubicin, high-dose daunorubicin *vs*. standard-dose daunorubicin) are warranted to confirm these preliminary results. If so, anthracycline scheme could modify *DNMT3A* exon 23 + patients outcome and could justify therapeutic stratification early at induction.

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ACUTE MYELOID LEUKEMIA IN PATIENTS AGED 70 OR OLDER. EXPERIENCE AT A SINGLE CENTRE

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Background. The management of old patients with acute myeloid leukemia remains controversial, specially in those cases affecting very old patients (aged >=70) in which the dilemma therapeutic abstention versus treatment (with low or high intensity schemes) is a major subject. **Aims.** We present the experience in our centre with this group of patients in the period 1990-2011. **Methods.** During the period of study 95 cases were diagnosed (relapses, FAB M3 cases and patients initially treated with 5-AZA were excluded). Patients were divided into 3 groups according to the treatment: no treatment (supportive treatment), low intensity treatment (low doses Ara-C: 10 mg/m^2/12h s. c. days 1-21) and high intensity treatment (adapted ICE: Idarubicin 10 mg/m^2 days 1 and 3; Ara-C 100 mg/m^2/12h days 1-3; Etoposide 100 mg/m^2 days 1-3). **Results.** The mean age of patients was 76,6 years (70-97); sex distribution was 47 males and 48 females; mean Karnofsky index was 70 (median 80; 30-100); 57 patients received treatment and 38 only did not; overall survival was 6,9 months (median 2; 0,03-90+), significant differences were observed in the mean overall survival between the treated and no-treated groups (9,9 vs 2,3 months respectively; p=0,017). In the low intensity group (31 patients) an overall response of 32,2% (6 CR, 4 PR, 14 NR and 7 not evaluable) was observed while in the high intensity one (26 patients) overall response was 53,7% (12 CR, 2 PR, 6 NR and 6 not evaluable); no statistical differences were observed between both groups considering all subgroups of response (p=0,13), or when these subgroups were grouped into PR+CR versus NR+Not Evaluable (p=0,11). Considering overall survival, no statistical differences were observed between the low and high intensity groups 7,1 (0,25-90+) vs 13,4 (0,25-90+) months (p=0,21) respectively. **Conclusions.** 1. - Overall survival in the treated group is higher than in the non-treated one, differences reached statistical significance. 2. - Comparing both arms of treatment no statistical differences were observed in the quality of response, though a higher proportion of complete responses were observed in the high intensity group (46% vs 19,3%, respectively), however, up to now, this circumstance has not contributed to a longer survival. 3. - No statistical differences have been observed in the overall survival between both groups of treatment.

1221

EVALUATION OF IMMUNE STATUS AT DIAGNOSIS AND IMMUNE MONITORING AFTER CHEMOTHERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Lymphocyte subpopulation is known to be associated with prognosis in patient with certain malignancies. The role of immune cells in hematologic malignancies, however, are not well studied. This study prospectively investigated whether differences in proportion of lymphocyte subset affect the survival or treatment outcomes in patients with acute myeloid leukemia (AML). **Methods.** Fourty-one patients with AML (M0, 1 patient; M1, 9; M2, 15; M3, 6; M4, 8; M6, 1; M7, 1; by FAB classification) were enrolled. The diagnosis of AML is made based on bone marrow study, immunophenotyping and cytogenetic analysis. We also underwent molecular study by Hemavision and PCR for FLT3, NPM1 and kit mutation. We measured lymphocyte subsets (T cells, helper/inducer T cells, suppressor/cytotoxic T cells, naïve T cells, memory T cells, regulatory T cells, NK cells and B cells) by multi-color flow cytometry with the peripheral blood from the patients at diagnosis and during complete remission (CR). **Results.** Patients are divided into 2 groups: Complete remission group (CR group, 30 patients) and non-responder group (NR group, 9 patients) after induction chemotherapy. We compared the lymphocyte subsets determined at diagnosis between 2 groups. The proportion of suppressor/cytotoxic T cells in CR group was higher than that of in NR group (p=0. 007). Patients were also classified into 3 groups as good, intermediate and poor prognosis groups by risk status based on cytogenetic and molecular abnormalities. In poor prognosis group, proportion of effecter memory T cells showed low level (p=0. 047) at diagnosis than that in other prognosis groups, but did not during complete remission. When poor prognosis group was divided into 2 categories as CR and NR, the CR group showed tendency of higher proportion in suppressor/cytotoxic T cells than NR group (p=0. 093). **Conclusions.** The high proportion of suppressor/cytotoxic T cells at diagnosis might contribute CR after induction chemotherapy, and it would be a strong marker for prognosis determination. The low proportion of effecter memory T cells at diagnosis is also associated with the poor cytogenetic or molecular risks in AML patients, and it could be one of the mechanism in leukemogenesis.

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CLOFARABINE IN COMBINATION WITH CYTARABINE (ARA-C) FOR TREATMENT OF RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA IN ADULT PATIENTS

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Introduction. Relapsed/refractory AML patients have a poor prognosis, with CR rates of 1%-30%, unless allogeneic hematopoietic stem cell transplantation (HSCT) is an available option. Although retrospective modeling studies have demonstrated the prognostic value of selected parameters, responses with salvage therapies remain still poor. It was previously established the activity of clofarabine plus cytarabine in AML relapse (clofarabine dosed once daily for 5 days with 40 mg/m2 followed 4 hours later by ara-C at 1 g/m2 per day). However, modifications of this combination in AML therapy of relapsed/refractory patients warrant further evaluation. **Aims.** To determine the efficacy and safety of clofarabine and cytarabine (Ara-C) in adult patients with relapsed or refractory acute myeloid leukemia (AML). **Methods.** Patients aged 35-66 years with refractory/relapsed AML were treated at the dose of clofarabine 30 mg/mq on days 1-5 + cytarabine 1000 mg/mq gg on days 1-5. We evaluated the complete remission rate (CRR), duration of remission (DOR) and overall survival (OS). Minimal residual disease (MRD) by molecular targeting was considered in all patients. **Results.** Seventeen patients received clofarabine 30 mg/mq on days 1-5 + cytarabine 1000 mg/mq gg on days 1-5 (their characteristics are summarized in Table 1), followed by gentuzumab therapy in only three patients. All patients had relapsed/refractory myeloid leukemia and had received multiple priors tharapies.

Table 1.

N	Age	Refractory/relapsed	Karyotype and molecular genetyc	Previous regimen (n.)	Response after Clofarabine/ARA-C	Toxicities	HSCT
1	55	refractory	47 XY,+8;	1	Refractory	Severe febrile neutropenia	n
2	41	refractory	48 XY, -21,+ 3 der(21)	1	Complete Remission	Severe febrile neutropenia	n
3	46	relapsed	46,XX/FLT3 ITD mutated	2	Refractory	Severe febrile neutropenia	n
4	55	relapsed	46, XY	2	Complete Remission	Severe febrile neutropenia	y
5	62	refractory	Complex karyotype, monosomy 7	1	Refractory	skin rash	n
6	66	refractory	47,XX,+8	1	Refractory	Severe febrile neutropenia	n
7	49	refractory	46 XX	2	Complete Remission	Severe febrile neutropenia	y
8	53	relapsed	46,XY	1	Died in induction	Skin rash	n
9	62	relapsed	46, XY	3	Complete Remission	Nausea, vomiting	n
10	48	relapsed	46,XY+8	3 + HSCT	Complete Remission	Nausea, vomiting	n
11	40	refractory	48,XY,+8	2	Died in induction	Nausea, vomiting	n
12	50	relapsed	46, XY	2	Refractory	Mucositis	n
13	42	refractory	46, XX/FLT3/ITD mutated	1	Complete Remission	Mucositis	n
14	61	relapsed	46, XY	1	Refractory	Severe febrile neutropenia	n
15	35	relapsed	46, XX	1	Complete Remission	Severe febrile neutropenia	y
16	40	relapsed	46, XY	1	Complete Remission	Severe febrile neutropenia	y
17	54	relapsed	46, XX/FLT3/ITD mutated	2 + HSCT	Complete Remission	hepatic transaminase elevations	n

Two pts had received a prior hematopoietic stem cell transplant (HSCT). Eight patients achieved a morphologic complete remission (CR);fourpatients went on

to receive allogeneic transplants after clofarabine/ARA-C salvage. The complete remission rate (CRR) was 52,94 %. The Median of Overall survival for all patients was 59 days (range 23-769), while the media of Overall survival (OS) was 158,41 days,and we estimated a duration of remission (DOR) as101,50 days in median (range 3-785), and 260 days in media (we calculated from the first day of remission). Treatment was complicated by neutropenic fever (n=9), grade III-IV mucositis (n=2), skin rush (n=2) grade II- III, hepatic transaminase elevations(n=1). Two patient died of sepsis during the induction. **Conclusions.** Combination treatment with clofarabine 30 mg/mq and ARA-C 1000 mg/mq in adults pts with refractory or relapsed AML resulted in an ORR of 52,94 %, and of the 8 patients who achieved a CR, four (50%) proceeded to HSCT (two are still alive and in complete remission). The safety profile is acceptable in this relapsed/refractory population, and our results are very similar to previous regimes using higher clofarabine dosages. More studies with this combination in adults are warranted.

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OVEREXPRESSION OF MN1 IS ASSOCIATED TO A LOWER RATE OF COMPLETE REMISSION AND AN INCREASE OF RELATIVE RISK OF RESISTANCE TO INDUCTION CHEMOTHERAPY IN AML OF INTERMEDIATE RISK

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Background. The presence of certain genetic disorders in Acute Myeloblastic Leukemias (AML) provides relevant prognosis information and allows to take therapeutic decisions. However, currently, up to a 40-50% of AML, according to the reported series, show a normal cytogenetic (AML-NK). In this subgroup of patients, submicroscope techniques have been shown to be useful in order to identify alterations (mutations or genes overexpression), which may help to clarify the prognosis and take decisions with these patients. NPM1, FLT3 or CEBPA are examples of these kind of disorders. In the last years, two studies about retrospective analysis of MN1 gene oveexpression in AML-NK group have been reported. They have shown that this gene overexpression has a negative impact on Overall Survival (OS), Progression Free Survival (PFS) and less rate of Complete Remisions (CR). **Aims.** To study the impact of MN1 gene overexpression in a group of 48 intermediate-risk AML patients in Complete Remission, who have been treated with al least one cycle of intensive chemotherapy, according to 3+7 scheme (idarubicin three days and cytarabine seven days).

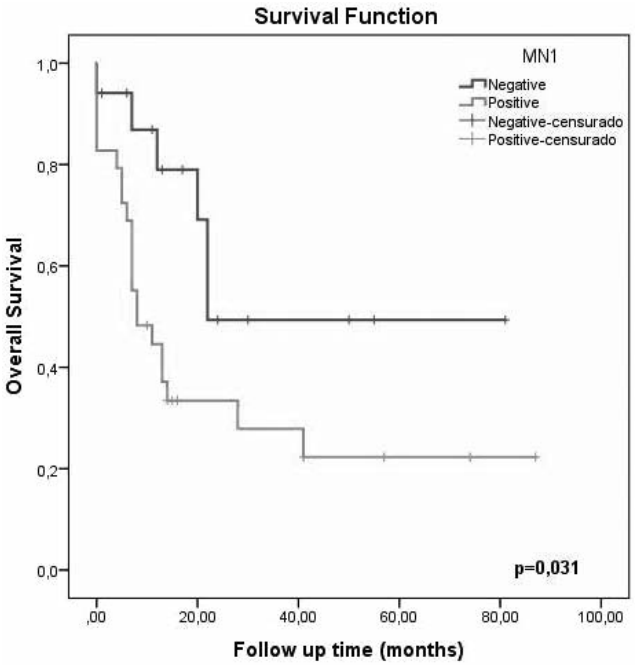

Figure 1.

Methods. We retrospectively study MN1 gene expression in bone marrow frozen samples of 48 AML patients, diagnosed between 2004-2011, with intermediate risk cytogenetic disorders according to MRC 1998 criteria. Median age was 54 years old and the range of age was from 15 to 75. Overexpression analysis was performed by Q-PCR. The cut-off was previously determined from the study of MN1 expression media in healthy bone marrow donors, determining overexpression from up to +2,5 SD. Normalization was performed using ABL as control gene. Relevant clinical data were reviewed in the clinical histories. Complete remission was studied after recovery from first chemotherapy cycle according to Cheson criteria. Statistical analysis was performed with the software SPSS 15. 0. **Results.** It was observed a rate of 48% of resistance to induction treatment in MN1 positive group against a 5. 9% in the MN1 negative group (P=0. 004 according to $\chi 2$ Test), with a Relative Risk (RR) of persistence of 8. 2 (95% CI 1. 2-57. 1). The survival study for the intermediate risk patients by Kaplan-Meier was of 27 months for the MN1 positive group and 42 months for the MN1 negative group (p=0. 031). However, we have to point out that there exist differences in the assignment of the consolidation therapy (32% Alo-BMT in the MN1 positive group against 21% in the MN1 negative group). **Conclusions.** 1. - In our serie, MN1 overexpression is associated with a higher rate of induction treatment resistance, with an increase resistance Relative Risk of 8. 2 (CI 1. 2-57. 1*).* Nonetheless, we believe that an increase in the sample size is necessary to define better the CI of the RR. 2. - We also observed a significative difference in overall survival (42 months vs 27 months p=0. 031), according to what has been published in the literature so far. Nevetheless, there exist differences in the consolidation treatment, and due to the small sample size, the age has not been taken into account as a risk factor.

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THERAPY-RELATED ACUTE MYELOID LEUKEMIA (T-AML) VERSUS AML WITH MYELODYSPLASIA-RELATED CHANGES AND DE NOVO AML: CLINICOBIOLOGICAL DIFFERENCES AND OUTCOME

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According to the WHO 2008 classification, t-AML is related to previous administration of chemotherapy and/or radiaton and comprises 10% of AML cases. Since it is considered a secondary leukemia, t-AML is often grouped along with AML evolving from myelodysplastic (MDS) or myelohyperplastic (MPD) syndrome, which is currently included in the AML with myelodysplasia-related changes (MDS/MPD-AML) entity. We retrospectively studied 359 patients who were diagnosed with AML (151 females, 208 males) with a median age of 50 (9-75) years. De novo (dn) AML was the initial diagnosis for 255 cases, while in 68 cases AML evolved after MDS or MPD (63 and 5 case respectively) and in 36 cases (14%) the disease was considered t-AML. Twenty five of these patients had received previous chemotherapy, 2 radiation and 9 cases were exposed to both cytotoxic therapies for various primary malignancies or autoimmune diseases; solid cancers: 9, NHL: 8, LH: 6, ALL: 2, AML: 3, myeloma: 3, aplastic anemia: 2, rheumatoid arthritis: 2, ITP: 1. The majority had received a combination of alkylating agents with topoisomerase II inhibitors and the median latent time for the t-AML diagnosis was 5 (1. 5-21) years. We investigated the clinocobiological characteristics and outcome of all patients, separating them in 3 groups: de novo AML, MDS/MPD-AML (sAML) and t-AML. Median age was 48 vs 62 vs 53 years respectively. White blood cell count (WBC) was higher in de novo AML group (median: 15600 vs 7000 vs 4900/µl respectively, p=0. 045) as well as MPO positivity in bone marrow blasts (median: 80% vs 39% vs 38% respectively, p=0. 038). Performance status and LDH were similar in all groups. Gytogenetic investigation revealed significant differences. The karyotypic prognosis was favorable in 15% vs 0% vs 6%, intermediate in 70% vs 65% vs 37% and poor in 15% vs 35% vs 57% of dnAML, sAML and tAML cases respectively. Achievement of complete remission (CR) along with overall survival (OS) was also different in the 3 groups. CR was achieved in 188/255 (74%) dnAML, 23/68 (34%) sAML and 12/36 (33%) tAML cases (p=0. 031). The 5-year disease-free-survival (DFS) was 31% vs 6% vs 24% (p=0. 046) and ten-year OS was 32% vs 3% vs 11% respectively (p=0. 004). In multivariate analysis WBC at diagnosis, dnAML and cytogenetic profile influenced DFS while significant factors for OS were dnAML, cytogenetic profile and age. In conclusion t-AML and MDS/MPD-AML seem to have similar response to treatment and OS. They differ in cytogenetic profile; the frequency of unfavorable cytogenetics is significantly higher in t-AML, yet favorable cytogenetics can be found. Both t-AML and MDS/MPD-AML share a different and worse outcome when compared to de novo AML.

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VALOR, AN ADAPTIVE DESIGN, PIVOTAL PHASE 3 TRIAL OF VOSAROXIN OR PLACEBO IN COMBINATION WITH CYTARABINE IN FIRST RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Background. The promising phase 2 activity/tolerability profile of vosaroxin with cytarabine in first relapsed or refractory AML (N=69) with median overall survival (OS) 7. 1 mo, combined complete remission rate 28% [complete remission (CR) 25%], median leukemia-free survival (LFS) 25 months, and 30-day all-cause mortality 3%, supported a phase 3 trial. **Aims.** VALOR (NCT01191801), a phase 3, randomized, controlled, double-blind trial, evaluates vosaroxin and cytarabine versus placebo and cytarabine in patients with first relapsed or refractory AML and incorporates an adaptive design. Primary objective is OS; secondary/tertiary objectives include CR rates, safety, event-free survival, LFS, and transplantation rate. **Methods.** Key eligibility criteria: persistent AML or first relapsed AML after 1 or 2 induction cycles that include at least 1 regimen of cytarabine with an anthracycline/anthracenedione; adequate cardiac, hepatic, renal function; and adults with no upper age restriction. Informed consent is obtained from all patients. Adaptive design: Base case assumed 40% improvement in median OS (hazard ratio, HR=0. 71), 90% power, 2-sided alpha of 0. 05. At the interim analysis (50% events), the Data and Safety Monitoring Board (DSMB) may recommend a 50% sample size increase from 450 to 675 evaluable patients if results indicate a larger sample size is required to reduce the risk of failing to confirm a clinically meaningful OS benefit (Mehta, Pocock, Stat Med 2010). In consideration of FDA and EMA guidance on Data Monitoring Committees and adaptive design with respect to trial integrity, operational bias, firewalls, and data confidentiality and archival, VALOR uses the Access Control Execution System (ACES®) to store, share, and archive confidential DSMB reports in a secure environment with an audit trail, and to facilitate communication between the DSMB and trial sponsor. The DSMB periodically reviews a combination of blinded and unblinded analyses while the study team remains blinded as specified in the DSMB charter. **Results.** VALOR is recruiting patients at over 100 sites in 14 countries in North America, Europe, and Australia/NZ. Enrollment opened Dec 2010; 183 patients enrolled through Dec 2011. The DSMB recommended VALOR continue as planned after reviewing safety data in Dec 2011. **Summary and Conclusions.** The key advantage of VALOR's innovative adaptive design is that the 50% increase in patient numbers is only called for at the interim analysis, if it is clear that this increase can substantially reduce the risk of failing to detect a clinically meaningful survival benefit. This novel methodology thus optimizes the chances of demonstrating clinical utility in the context of a disease as complex and heterogeneous as AML. Enrollment opened Dec 2010; 183 patients enrolled through Dec 2011. The DSMB recommended VALOR continue as planned after reviewing safety data in Dec 2011. The outcome of the interim analysis is anticipated in the third quarter of 2012.

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CHARACTERISTICS AND OUTCOME OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE MULTIPLE-CENTER ANALYSIS

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Introduction. Acute myeloid leukemia (AML) is more frequent in elderly patients, and the prognosis is very poor. Currently, there is no established standard treatment since the survival of patients treated with intensive chemotherapy is 10% at 3 years. In addition, most patients are not candidates for intensive treatment due to age, comorbidity or other biological characteristics. **Methods.** We conducted a retrospective analysis of 167 consecutive patients 65 years of age or older, diagnosed for AML between January 2007 and December 2010, in several health departments in Spain, and without selection bias. This was done in order to analyze the biological characteristics of leukemia, and patient characteristics and responses to different treatments. Variables related to patient and disease characteristics were compared among groups using *t* Student test for categorical variables and Krukal-Wallis for continuous variables. Cox regression analysis was used to explore the relationship between survival and explanatory variables. Survival was calculated utilizing Kaplan-Meier and long-rank test. **Results.** Total subjects were 91 men and 74 women. Median patient age was 75 years (range, 65-92 years). Forty-eight patients (28. 7%) had a secondary AML and 35 (21%) a previous myelodysplastic syndrome. In 83 patients (50%) cytogenetics was not performed or not valuable. Cytogenetic groups according to MRC criteria were as follows: favourable 5 (6%), intermediate 35 (42%) and adverse 43 (52%) -see Table 1-. In multivariable analysis, PS <= 2, intensive chemotherapy and 5-AZA treatment were found to be statistically significant for survival.

Table 1. Clinical characteristics and outcomes according to type of treatment.

Variables	Palliative (n=87)	Ara-C (n=10)	AZA (n=19)	Chemotherapy (n=51)	*p*
Age (median)	80 [65-92]	75 [67-81]	74 [66-86]	70 [65-78]	0. 000
Sex (men/women)	47/40	6/4	11/8	29/22	ns
PS >2	44. 3%	22%	15. 8%	5. 9%	0. 000
Comorbidities	1 [0-6]	2 [0-4]	1 [0-5]	1 [0-5]	ns
I Sorror >= 2	59. 7%	60%	69%	31. 8%	0. 02
Hb at diagnosis (g/dL)	9 [4. 5-12. 4]	9. 1 [5. 7-14. 0]	8 [6. 6-13. 9]	8. 8 [4. 5-12. 4]	ns
Leucocytes (x10^9/L)	11. 5 [1-357]	16. 5 [2-168]	4. 0 [1-47]	5. 7 [0. 7-178]	ns
Platelets (x10^9/L)	57 [6-2463]	73. 5 [10-321]	72 [19-459]	50 [4-242]	ns
PB blasts (%)	18 [0-95]	26 [0-95]	6 [0-76]*	29 [0-97]	0. 006
BM blasts (%)	45. 5 [12-99]	52 [28-92]	30. 5 [3-90]	52 [20-99]	ns
Secondary AML	34. 5%	50%	31. 6%	13. 7%*	0. 025
Favorable cytogenetic	0%	0%	0%	9%	-
Intermediate cytogenetic	17. 2%	50%	10. 5%*	25. 5%	0. 000
Adverse cytogenetic	18. 4%	0%	47. 4%*	35. 2%	0. 000
Unknown cytogenetic	64. 4%	50%	42. 1%	29. 4%*	0. 000
CR (%) Cheson 2003	0%	0%	0%	57%	-
Survival (median in months)	1. 6 [1. 2-2]*	4. 1 [0-8. 3]*	7. 4 [1. 7-13. 1]	8. 2 [5. 6-10. 8]	0. 000

Conclusions. Patients aged over 65 years, diagnosed with AML are a group with a very poor prognosis. There is an increased prevalence of patients with secondary AML, poor cytogenetic profiles and high rates of comorbidities. Although the clinical and biological characteristics of the intensive chemotherapy group are more favourable, overall survival was not significantly better than that for the group treated with 5-AZA. Further research is needed to establish optimal management and improve outcomes of elderly patients with AML.

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TWO STEPS FLOW-CYTOMETRIC DIAGNOSIS OF ACUTE LEUKEMIA: SINGLE CENTER EXPERIENCE

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Background. Flow cytometry analyses are powerful diagnostic tool for acute leukemia and their classifications in different acute myeloid leukemia (AML) from acute lymphoblastic leukemia (ALL) entities. Inspired by a resent published paper concerning the distinct CD45/SSC panel associated with AML and ALL cases we analyzed our lab data-base in order to make cost-effective and more efficient our diagnostic work-up for acute leukemia. **Methods.** We retrospectively analyzed 140 consecutive cases of acute leukemia that were diagnosed in the last two years at the University Clinic of hematology-Skopje. We correlate the blast distribution patterns in the CD45/SSC panel with the morphology and the detailed immunophenotype. The diagnosis of acute leukemia was made by standard morphological examination and cyto-chemical analyses of bone marrow smears according to the criteria established by FAB Cooperative Study Group and confirmed by immunophenotyping of bone marrow aspirates and/or peripheral blood samples following the criteria of the European group for the immunological Classification of acute leukemias (EGIL). Flow-cytometric analyses were performed by using the FAXS Canto II BD flow cytometer analyzer. Acquired data were analyzed with the software FACSDiva version 6. 1. 2 by using CD45 gating strategy. Slightly modified panel of monoclonal antibodies (McAb) against myeloid- and lymphoid-associated antigens as suggested by the EGIL was utilized. The samples contained more than 20% of blast cells (most of which had more than 50%). Antigen expression was considered positive if 20% or more blats cells reacted with the particular antibody, except reactivity of blast cells with anti-MPO which is considered positive if 10% or more mononuclear cells are MPO positive **Results.** Our results showed that blast distribution patterns in the CD45/SSC panel in correlations with the morphology allows primary provisional distribution of acute leukemia cases in the three groups *AML, ALL,* and one *indeterminate group which* guides to implementation of specific immunological analyses in each group with *modified AML, ALL,* and *indeterminate* flow panels. Using this two step approach, we have efficiently and correctly diagnosed almost all acute leukemia cases. Our analysis also determined the minimal numbers of immunological markers needed for the lineage assignment of acute leukemia. **Conclusions.** Our two steps immunological analyses of acute leukemia cases maintained diagnostic accuracy while significantly improved the cost efficacy of acute leukemia diagnostic work up by reducing reagent use, labor, and time.

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AGE SPECIFIC CYTOGENETICS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA IN CHINESE POPULATION

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Background. The clonal chromosomal abnormalities observed in the leukemia cells of patients diagnosed with acute myeloid leukemia (AML) are the hallmark of AML. Different mechanisms may lead to different types of genetic alternations. The mechanisms might occur at different frequencies over lifetime. Age is an important factor to be considered when investigating the etiology of a disease as it can provide clues to its pathogenesis. Both age and cytogenetics are critical for acute myeloid leukemia (AML), and the relationship between these two factors were reported in several previous studies. However, little is known about Chinese population, and there are also limited data on the cytogenetics of AML in children. **Aims.** To observe the distribution of cytogenetic subgroups in Chinese population with AML of both children and adults. **Methods.** In present study we analyzed 684 patients with *de novo* AML. The patients were divided into six age groups, group I (≤16 years), II (16-30 years), III (30-40 years), IV (40-50 years), V (50-60 years), VI (≥60 years) according to age factor. The cytogenetically grouping was performed as normal, balanced (including translocations and inversions), monosomal (two or more distinct autosomal chromosome monosomies or one single autosomal monosomy in the presence of structural abnormalities), complex (≥3 clonal aberrations) and unbalanced karyotype (get or loss in genetic quantity, excluding monosomal and complex karyotype). All participating patients gave informed consent prior to enrollment into the studies. **Results.** The frequency of normal, balanced, monosomal, complex and unbalanced karyotype were 39. 62%, 32. 89%, 2. 92%, 2. 34% and 22. 22%, respectively. The distribution of normal and balanced karyotype showed age specific characteristics. The frequency of normal karyotype was increasing from the group I (30. 2%) to the group VI (48. 7%) ($\chi 2$=15. 459, P=0. 009). The incidence rate of balanced karyotype decreased from the group I (37. 7%) to the group VI (17. 7%) ($\chi 2$=22. 801, P<0. 001). The incidence rate of unbalanced karyotype remained static across the different age groups. There was no evident age specific tendency was detected for both monosomal and complex karyotype groups because of the low incidence of these aberrations. No significant difference was observed in frequency of abnormal karyotype between children and adults (69. 8% versus 59. 6%) (χ^2=2. 136, P=0. 144), and the distribution of cytogenetic subgroups was not evidently different ($\chi 2$=8. 577, P=0. 073) as well. t(8;21) and t(15;17) occurred at the highest incidence in children and adults, respectively. **Conclusions.** The different age profiles of the cytogenetic subtypes may indicate the different mechanisms of the pathogenesis of AML, which may also offer beneficial information for etiological research and development of new treatment.

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PROGNOSTIC SIGNIFICANCE OF WT1 EXPRESSION AT DIAGNOSIS AND END OF INDUCTION IN EGYPTIAN ADULT ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Acute myeloid leukaemia (AML) is the most common leukaemia in adults, only about one-third of these patients remain free of disease for more than five years. Wilms' tumor (WT1) gene encodes a transcription factor important for normal and malignant haematopoiesis. Its overexpression has been reported in the majority of AML patients at diagnosis and has been evaluated as prognostic and minimal residual disease (MRD) marker. **Aims.** To assess the prognostic significance of WT1 overexpression at diagnosis and to evaluate its use as MRD marker at the end of induction. **Patients and Methods.** WT1 overexpression was evaluated in sixty eight adult AML patients at diagnosis using quantitative real time RT-PCR. The impact of WT1 transcript reduction at the end of induction was evaluated in eighteen patients in CR in whom the initial level of transcript at diagnosis was sufficient to follow-up using 2 log reduction. Patients received Doxorubicin and Cytosine arabinoside (3 and 7 regimen) for induction. **Results.** WT1 overexpression was detected in 51/68 (75%) of patients. No significant associations were encountered between WT1 overexpression at diagnosis and other prognostic factors. Complete remission was achieved in 74% of patients with WT1 overexpresssion compared to 80% of patients with normal levels (p=0. 5). No significant associations were encountered between WT1 overexpression at diagnosis and DFS or OS (p=0. 6 and 0. 3, respectively). At the end of induction, the median duration of DFS in patients achieving ≥2 log reduction was not reached compared to 5 months (range: 2. 1-7. 9m.) in those attaining <2log reduction (p=0. 2) (Figure 1).

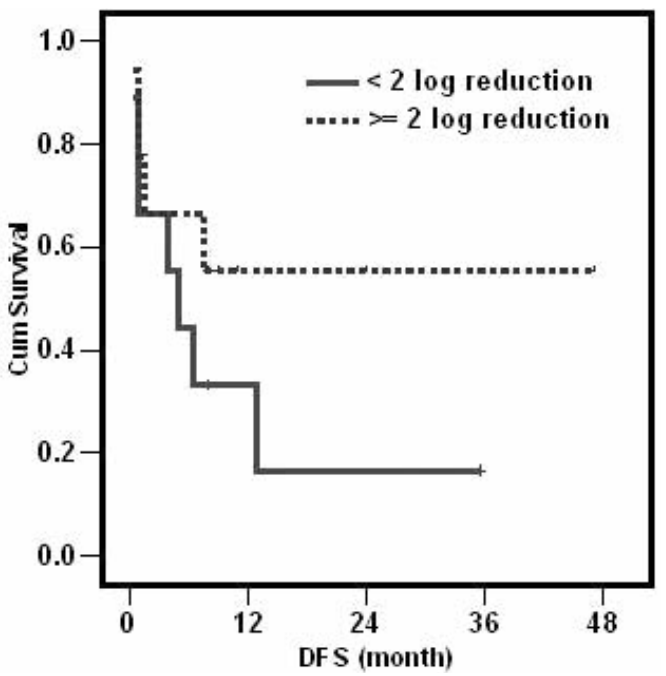

Figure 1. DFS in relation to ≥2log reduction at the end of induction (p=0.23).

The median duration of OS in patients achieving ≥2log reduction was 13 months (range: 0-33. 3m.) compared to 7. 5 months (5. 4-9. 6m.) in those attaining <2log reduction (p=0. 3). The survival at one year in patients achieving ≥2 log was

double the group with <2log reduction 66. 7% compared to 33. 3%. **Conclusions.** Our results, although not reaching the level of significance, which could be due to the small sample size, suggest that early assessment of the WT1 transcript level at the end of induction in patients in CR may have an impact on clinical outcome and may thus be a useful marker for risk stratification especially in patients lacking disease specific marker. Its applicability must be evaluated in a larger cohort of patients.

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INTERNAL TANDEM DUPLICATION (ITD) MUTATIONS OF THE FLT3 RECEPTOR AND CXCR4 EXPRESSION IN DE NOVO ADULT ACUTE MYELOID LEUKEMIAS (AML)

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Introduction. Internal tandem duplication (ITD) mutations of the FLT3 receptor are associated with a high incidence of relapse in acute myeloid leukemia (AML). It is also known that the complete immunophenotypic characterization contributes to define the prognosis of de novo adult AML. Stromal cell-derived factor-1 (SDF-1)is a homeostatic chemokine that is constitutively secreted by marrow stromal cells. SDF-1 signals through CXCR4, which plays an important role in hematopoiesis, development and organization of the immune system. Prognostic impact of CXCR4 expression levels on the neoplastic cells has been demonstrated in breast cancer, renal cell cancer and AML. **Aims.** We investigated the expression of the chemokine receptor CXCR4 on bone marrow blast cells in a group of adult de novo AML with FLT3-ITD. **Methods.** We have observed 45 young adult de novo AML patients (median age: 44 years, r: 22-62) in the last 4 years, who presented FLT3-ITD-positive AML blasts in bone marrow blood. On the basis of FAB classification the patients were considered LMA-M5 (17 pts.), LMA-M2 (12 pts.), LMA-M4 (6 pts.), LMA-M1 (7 pts.) and LMA-M0 (3 pt.). 12 patients showed complex chromosomal anomalies; 19 patients presented hyperleukocytosis (WBC> 40x10(e)9/L). The clinical outcome was that one of a "high risk"AML; at the present only two patients are still alive in CR (+40 months and +46 months). **Results.** We found at the diagnosis, in all cases, an high CXCR4 expression on leukemic blasts, as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5. **Conclusions.** Several studies have shown the prognostic significance of the expression of differentiation myeloid markers at the diagnosis of adult de novo AML. However, specific immunophenotype expression patterns associated with internal tandem duplication (ITD) mutations of the FLT3 receptor are still unknown. Further studies are warranted to confirm the correlation between FLT3-ITD and "CXCR4 over-expression" immunophenotypic pattern.

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ADULT ACUTE MYELOID LEUKEMIA IN FIRST RELAPSE - SUPPORTIVE VS. SALVAGE THERAPY

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Introduction. For adult patients diagnosed with Acute Myeloid Leukemia (AML) who achieve complete remission (CR), maintaining remission is the primary goal. However, the majority of patients will relapse. The probability of relapse depends of numerous factors including age and cytogenetics at diagnosis. Despite the progress in understanding the pathophysiology of this disease, the prognosis after AML relapse is still very poor. Frequently the clinician is faced with the decision to intensively treat a relapsed AML patient, with all the inherent complications' risk, or to provide the best supportive care in view of a quality-of-life benefit. **Aims.** To compare the survival after relapse between adult AML patients in first relapse who received therapy without achieving CR and patients given supportive care. **Methods.** Retrospective review of the clinical files of adult patients diagnosed with AML, excluding all cases of Acute Promyelocytic Leukemia, between January 1998 and December 2010 at our Institution (268 patients). Of the 246 patients submitted to induction therapy, 202 achieved CR with relapse occurring in 88 patients. At the time of 1st relapse, 27 patients were given only supportive care (group A) and 61 were submitted to intensive treatment; of those 28 achieved a 2nd CR and 33 didn't respond to therapy (group B). Survival was compared between groups A and B and was measured from the time of relapse. Statistical analysis was performed with SPSS®Statistics v19. **Results.** Comparing both groups, A and B, patients on the supportive group were older (median age: 61 years vs. 47. 5 years, p= 0. 03) and relapsed earlier (median time to relapse: 3. 95 months vs. 10. 65 months, p=0. 082). Distribution of gender, performance status and karyotype at diagnosis was similar in both groups; no patient presented favorable cytogenetic risk. The most frequent chemotherapy regimens used were SWOG-9126 (33. 3%), and VC (Etoposide+Carboplatin; 33. 3%). Overall, median survival after relapse was 1. 7 months in group A and 5. 9 months in group B (p=0. 003), which represented a survival benefit of 4. 2 months for patients who received chemotherapy. On multivariate analysis, age (p=0. 022) was the only independent prognostic factor identified. Subgroup analysis was performed in order to identify those patients who achieved the most benefit from salvage therapy. We identified age ≤60 years (median survival: 1. 6 months in group A vs. 6. 8 months in group B, p= 0. 014), time for relapse >12 months (median survival: 1. 5 months in group A vs. 7. 6 months in group B, p=0. 007) and intermediate risk cytogenetics (median survival: 1. 6 months in group A vs. 5. 9 months in group B, p=0. 01) as the groups with superior survival advantage. **Conclusions.** We conclude that there is a survival benefit associated with receiving salvage therapy in first relapse, in spite of failure to achieve CR, when compared to supportive treatment only. That advantage might be related to a reduction in the proliferation of malignant cells and/or an improvement in "functional"cell counts. However, this was a retrospective analysis and we admit the results may reflect a bias related to the selection of the more "fit"patients to receive treatment.

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EXPERIENCE OF A 5-DAY SCHEDULE OF AZACITIDINE IN A SINGLE-CENTRE ACUTE MYELOID LEUKEMIA POPULATION

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Background. Acute Myeloid Leukemia(AML) is the most common type of acute Leukemia occurring in adults. First line Intensive chemotherapy would be the best treatment option but for some population, as elderly patients, who have poor performance status and other recognized poor prognostic factors may not be the most suitable option. Similarly, AML refractory or patients with an early relapse after induction, have a poor prognosis and limited therapeutics options. For both cases, preliminary studies with 5-Azacitidine seems to be an active and well tolerated drug pending to be corroborated in further prospective studies. **Aims.** To describe our experience with 5-Azacitidine in unfit or no candidate for intensive chemotherapy and relapse/resistant AML patients and to compare it with previous data reports in the literature. **Methods.** We include in these study all AML patients (and 4 high-risk MDS) treated in our center with 5-Aza. The protocol used was 75 mg/m2/day intravenous for 5 consecutive days every 28 days. Data of responses were described according to IWG criteria. **Results.** 20 patients were included, 16male/4Female, with a median age of 73. 5 years (39-81) with 16 patients(80%) >65 years. According to WHO criteria, 16 were AML (4 unfit untreated, 12 relapse/resistance) and 4 MDS (all four with high risk IPSS and severe transfusion dependence, 3 unfit-untreated, 1 relapse/resistance). 3/20 patients are separately reported because they began 5-Aza as a consolidation/maintenance in Complete Remission(CR) after a cycle of chemotherapy induction. Median pre-treatment Bone Marrow blasts percentage in AML patients was 31%(6-90). Karyotype was evaluable in all patients, 11(74. 8%) adverse-risk, 6(35. 2%) intermediate-risk). A total of 83 treatment cycles were administered with a median of 3 cycles (1-18) (all patients included, at least completed 1 cycle). After a median follow up of 8. 3 month (1-21), 7/17(41. 1%) had some kind of response (CR/partial remission/hematology improvement(HI) and Stable disease(SD). With 3 CR(17. 64%) and 4 SD(23. 5%) We did not find any PR or HI. The median response duration of CR was 5 month (3-9). The median overall survival(OS) time of the whole cohort was 4. 9 month (0. 4-23,3). OS in the adverse and intermediate-risk Karyotype group was 4. 35 and 6,6 month respectively and in the unfit-untreated and relapse/resistance patients was 5. 03 and 4. 9 month. In the 3 patients who began 5-Aza in CR after induction therapy the median OS and EFS was 25. 3 and 10. 8 month respectively. **Conclusions.** As this is a retrospective study with a small simple size and heterogenety among the patients, it is not posible to stablish statistically significant results. Taking this facts into account, we can conclude that our OS, CR, DFS data and the difference by cytogenetic risk group, coincide with what have been published in the literature. In the same way, OS and DFS data obtained in our 3 patients on maintenance treatment are also similar to what have been described in similar AML patients after induction treatment. Therefore, these preliminary data encourage us to continue in this line of treatment, as well as to widen and improve our study in this poor prognosis patients with limited treatment options.

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PATTERN OF DISSEMINATED INTRAVASCULAR COAGULATION IN ACUTE PROMYELOCYTIC VS OTHER SUBTYPES OF ACUTE MYELOID LEUKEMIA

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Background. Acute promyelocytic leukemia (APL) is a specific subtype of acute myeloid leukemia (AML) with distinctive morphology, molecular presentation, clinical course, and treatment. Up to 90% of APL patients present with hemorrhagic complications due to disseminated intravascular coagulation (DIC), which constitutes one of the most serious and often lethal aspects of the disease. When APL is suspected, initiation of all-trans retinoic acid (ATRA) is recommended, without waiting for molecular confirmation. However, published criteria are lacking for differentiating unconfirmed APL from other AML subtypes in the setting of DIC. **Aim**Herein, we report our comparison of the DIC pattern in APL with that of other AML subtypes. **Methods.** From January, 2005 to November, 2011, a retrospective analysis of patients diagnosed with APL (n=10) and other subtypes of AML (n=14) was conducted to establish diagnostic criteria for DIC. **Results.** INR (1. 44 vs 1. 23, *p*=0. 044) and FDP (79. 42 μg/mL vs 21. 06 μg/mL, *p*=0. 033) levels were higher, as were neutrophil fractions (65. 23% vs 28. 07%, *p*<0. 001), and fibrinogen (134. 92 mg/dL vs 354. 86 mg/dL, *p*<0. 001) was lower in patients with APL compared with other AML subtypes. Accordingly, our threshold values were as follows: INR ≥1. 35, FDP ≥27 μg/mL, neutrophil fraction ≥50%, and fibrinogen ≤220 mg/dL. Given that at least three of these criteria are met, the diagnostic sensitivity and specificity for APL are 80. 0% and 92. 86%, respectively. These markers may thus be characteristic of APL and help support a presumptive diagnosis. **Conclusions.** APL may be differentiated from other subtypes of AML by core markers of DIC (INR, FDP, fibrinogen, and neutrophil fraction), for which we suggest new diagnostic thresholds. Despite its small scale, our study offers added support for early initiation of ATRA, prior to PML-RARA molecular confirmation.

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FLUDARABINE BASED CHEMOTHERAPY REGIMENS FOR HIGH RISK ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Acute myeloid leukemia (AML) patients older than 60 years have a poor prognosis due to many unfavorable factors: comorbidities, poor tolerance and elegibility to intensive treatment, multiple chromosomal abnormalities, higher incidence of secondary diseases, low response rate to treatment. Secondary or refractory/recurrent AML as well, represents an high risk subset because of a particularly aggressive disease, multidrug resistance expression resulting in poor response to treatment. In that subset of patients, standard induction regimens based on cytarabine and daunorubicin association showed low remission rates and poor outcome. Moreover, many of these patients are not eligible for transplant procedures due to age or to toxicity. The association of fludarabine and cytarabine as induction therapy seems to offer an effective treatment in AML patients with high risk disease even if few data have been published until now. We describe thirty-one cases of patients affected by high risk AML treated with combined regimens with fludarabine and cytarabine. **Patients and Methods.** From November 2005 to December 2011 in our Clinical Hematology Unit we observed 31 patients with high risk AML eligible for aggressive induction chemotherapy (17 males, 14 female; median age 67 years, range 34-74); 14 patients aged > 60 years presented *de novo* AML, 7 patients were affected by AML secondary to myelodysplastic syndrome (MDS), 1 patient showed AML secondary to mitoxantrone treatment for multiple sclerosis, 9 patients showed refractory/recurrent disease. Patients were all treated with fludarabine based regimens: 8 with FLA regimen (Fludarabine 30 mg/m^2 day 1-5, Ara-c 2 g/m^{2}day 1-5), 19 with FLAN regimen (Fludarabine 30 mg/m^2 day 1-5, Ara-C 2 g/m^2 day 1-5, Mitoxantrone 10 mg/m^2 day 3-5), and 4 with FLAI regimen (Fludarabine 30 mg/ m^2 day 1-5, Ara-C 2 g/ m^2 day 1-5, Idarubicin 10 mg/ m^2 day 1,3,5). **Results.** After induction treatment, 21/31 patients obtained a complete remission (CR) (67. 6%), 5 (16%) were refractory and 5 (16%) died during treatment because of infection. 10 patients (47,6%) who achieved CR, relapsed after a median time of 8 months (range 2-17 months). 8 patients (38%) died in first CR: 6 during consolidation treatment (4 due to sepsis, 1 due to brain hemorrhage and 1 due to invasive candidiasis) and 2 before consolidation treatment because of infection. Currently, 3 patients are in continuous complete remission from respectively 69. 5, 64. 5 and 14 months and 1 patient is in second CR with a follow-up of 37 months from diagnosis. Median overall survival (OS) is 4 months (range 0. 1 - 66. 6+), with 24% of patients projected to be survivor at 24 months, while median disease free survival (DFS) is 8. 4 months (range 0. 9 - 70. 6+), with 19. 4% of patients at 24 months. **Conclusions.** In our experience combined regimens with fludarabine and citarabine represent an effective induction treatment for AML patients with high risk disease; unfortunately, infections still represent a primary cause of death especially in heavily pretreated patients.

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OUTCOME OF FLAG REGIMEN IN THE TREATMENT OF REFRACTORY OR RELAPSED ADULT ACUTE MYLOID LEUKEMIA

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Background. A large proportion of adult patients with acute myeloid leukemia (AML) relapse after treatment, and some of them are resistant to primary induction chemotherapy. Over the past decade emphasis has been made in the development of regimens containing fludarabine, combined with cytosine arabinoside for the treatment of refractory/relapsed acute leukemias. **Aims.** The aim of this study was to evaluate the efficacy and toxicity of fludarabine, high dose cytarabine, and granulocyte colony stimulating factor in refractory or relapsed acute myloid leukaemia. **Methods.** Between January 2008 and February 20011, 23 adult patients older than 16 years, with primary refractory or relapsed AML, with exclusion of promyelocytic leukemia, were included in this study. Absence of heart, liver, or kidney damage, as well as no active infection, were required for inclusion in the trial. Adequate heart function was assessed by echocardiography(left ventricular ejection fraction ≥50%) and electrocardiogram. Written informed consent was obtained from all patients. Morphologic leukemia subtypes wereevaluated on bone-marrow smears according to standard procedures, using the revised FAB criteria. Treatment protocol consisted of Fludarabine 25 mg/m2/d (d1-5) i. v. as a 30-minute infusion, then 4 hours later Ara-C 2 g/m^2/d (d1-5) i. v. as a 4-hour infusion; G-CSF 300 microgram/day was given subcutaneously from day zero until absolute neutrophil count (ANC) >1x 10^9/L. **Results.** There were 14 men and 9 women with a median age of 32 (range 21-44) years. ten patients had primary refractory disease, and 13 were in first relapse. Twelve (52. 1%) patients achieved complete remission (CR) following salvage therapy, whereas 10 (43. 4. 5%) patients were refractory, and one patient died in aplasia due to infection. In patients achieving remission, the median time to reach absolute neutrophil count (ANC)more than 1×10^9/l was 24 (range 20-28) days from the start of chemotherapy, Platelet level of more than 20×10^9/l was achieved in a median time of 23 (range 19-25) . Nonhematological side effects, consisting mainly of mucositis (18/23 or 78. 2%) and transient liver toxicity increase (10/23 or 43. 4%), were generally tolerated. Of twelve patients who achieved CR 3 patients received a second course with FLAG, and 9 patients stayed without treatment (allogeneic stem cell transplantation was not available) while two did not reach that stage due to early relapse from CR. The median overall survival (OS) for all 23 patients was 4. 5 (range 1-38) months. **Conclusions.** FLAG is a good choice in cases with refractory or relapsing acute myeloidleukemia for salvage chemotherapy. High efficacy and a low-toxicity profile are preferable properties of this regimen, and this regimen has been found to be useful for cytoreduction, especially in candidates for allogeneic stem cell transplantation

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NPM1 AND FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA (AML)

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Background. Acute Myeloid Leukemia (AML) is a disease with marked heterogeneity in both response to therapy and survival. The molecular analysis resulted in the emergence of new prognostic factors, including gene mutations in FLT3 (Fms-like tyrosine kinase 3) and NPM1 (nucleophosmin 1). The presence of these gene alterations may exert profound effects on outcome of disease. **Aims.** To investigate the clinical and biologic characteristics and the prognostic impact of NPM1 and FLT3 mutations in AML. **Materials and Methods.** One hundred and thirty patients with AML were consecutively admitted in our hospital between October 2008 and March 2011. Were included patients with de novo AML and secondary to myelodysplastic syndrome. Ninety one patients were candidates to intensive chemotherapy. Only 71 of 91 patients were analyzed for the presence of FLT3 and NPM1 mutations. Patients were treated with EORTC Protocols AML12/AML17, AIDA GIMEMA protocol for acute promyelocytic leukemia and 1 patient with ELAM-02 pediatric protocol, followed by consolidation chemotherapy and allogeneic or autologous transplantation. **Results.** Median age was 51 years (range,16-78) and 44 (62. 0%) were

females. FLT3 mutation was found in 18 (25. 4%) patients, 13 with FLT3 internal tandem duplication (FLT3ITD) mutation and 5 with FLT3 Tirosine Kinase Domain (FLT3TKD). NPM1 mutation was found in 14 (19. 7%) cases. Nine of these (64. 3%) were also FLT3+ (p=0. 001). Median leukocyte count was significantly higher in patients with NPM1 mutation (81. 04x10^9/L vs. 7. 30x10^9/L, p=0,003) and FLT3 mutation (64. 70x10^9/L vs. 5. 45x10^9/L, p=0. 000). Median *lactate dehydrogenase* (LDH) was higher in FLT3+ patients (p=0. 03) and NPM1+ patients (p=0. 05). FLT3+ patients had significantly higher blasts counts (81% vs. 60%, p= 0. 008), but not NPM1+ patients. We observed a trend of FLT3+ patients being classified with FAB subtype M1, M3v and M4/M5 (p=0. 07) and a significant association between NPM1 mutation and subtypes M1, M2 and M4/M5 (p=0. 02). FLT3 mutation presented more frequently in patients with normal karyotype and t(15;17) or PML-RARA (p=0. 92). Normal karyotype was observed in thirteen (92. 9%) of the NPM1+ patients (p=0. 01). To assess the prognostic impact, we analyzed the intermediate cytogenetic group of patients (n=38), with a median follow-up of 12 months (0-31). One patient died before start chemotherapy. Patients were divided in group 1 (NPM1+, FLT3ITD-), 2 (FLT3ITD-, NPM1-), 3 (FLT3ITD+, NPM1+) and 4 (FLT3ITD+, NPM1-). No statistical significance was found in Disease Free Survival (DFS) rates, with a 2-year DFS of 66. 7%, 27. 6% and 25. 0% in group 1, 2 and 3, respectively (p=0. 10). None of group 4 patients achieved complete remission. The 2-year Overall Survival (OS) was significantly higher in group 1 (83. 3%), than group 2 (34. 3%), 3 (28. 6%) and 4 (0%) (p=0. 002). **Summary and Conclusions.** Our data confirm that the presence of FLT3 and NPM1 mutations is associated with specific clinical characteristics in AML patients, like higher median leukocyte count and LDH. *FLT3ITD* mutation was associated with an unfavorable outcome and *NPM1* mutation, in the absence of FLT3ITD, may be a favorable prognostic factor for OS.

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CLINICAL CHARACTERISTICS AND ANALYSIS OF NONLEUKEMIC MYELOID SARCOMA: A REPORT OF 13 CASES

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Background. Myeloid sarcoma (MS) is an extramedullary mass composed of immature myeloid cells. The tumor may involve any part of the human body, especially the skin, bone and lymph nodes. On rare occasions, MS manifests before the onset of AML, in which case it is often misdiagnosed as malignant lymphoma. An accurate diagnosis of nonleukemic MS is important for effective therapies. **Aims.** To analyze the clinical characteristics, immunohistochemistry, cytogenetic findings, treatment approaches of myeloid sarcoma (MS), and assess the prognosis. **Methods.** Thirteen cases of nonleukemic MS were enrolled in this study. Histologic and immunohistochemical examinations were performed on biopsies or surgical specimens for MS diagnosis. All patients underwent a complete history, physical examination, and laboratory tests included peripheral blood cell count, bone marrow aspiration and biopsy. Clinical characteristics, pathological findings, treatment response and prognosis were analyzed. **Results.** The male-to-female ratio was 1:1. 6. Majority of patients (11/13, 84. 6%) received diagnosis between the ages of 20 and 42. The most sensitive immunohistochemical biomarkers were myeloperoxidase (12/13, 92. 3%). Chromosomal abnormalities were only observed in one cases with the t (8; 21) (q22; q22) translocation. All patients received systemic anti-AML chemotherapy. The median survival time was 20 months (range, 4-63 months), of which four cases (4/13, 30. 8%) progressed to AML within 16 months (range, 6-62 months). **Conclusions.** Accurate initial diagnosis and aggressive treatment play a key role on improving the prognosis of nonleukemic MS. Anti-AML chemotherapy is still the first line treatment. The treatment choice of refractory and relapsed MS is still difficult. The standardized and unified strategies are needed to develop for effective treatment, by expanding the case samples and designing prospective studies.

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ACUTE MYELOID LEUKEMIA-TYPE CHEMOTHERAPY REGIME INDUCES COMPLETE REMISSION OF BLASTIC PLASMACYTOID DENDTRITIC CELL NEOPLASM

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Background. Blastic plasmacytoid dendritic cell (BPDC) neoplasm is a rare and highly aggressive hematological malignancy. Formerly, it was known as blastic natural-killer cell lymphoma or CD4/CD56 hematodermic neoplasm because it almost always presents with skin lesions, and often involves lymph nodes and bone marrow. Although conventional chemotherapy leads initial to high response rates, relapse rates remain very high, then with chemotherapy-resistant disease. **Aims.** Several treatment protocols were suggested with controversial results. However, long-term remission and survival rates appear to be achieved only by hematopoetic stem cell transplantation. Herein we report three patients, which were treated in our hematologic department in 2011 with different clinical manifestations. **Case Report.**Two patients presented with painless reddish erythematous skin plaques. The patients denied fever, night sweats or weight loss. Complete blood count was normal. After several months of topical treatment with corticoids no improvement could be observed. Repeated skin biopsies were performed and revealed a diffuse lymphomatous infiltrate demonstrating a BPDCN. A computed tomography scan of the chest and abdomen as well as bone marrow biopsy revealed no further manifestations. Third patient presented initially with fever and pancytopenia. Bone marrow aspiration was performed. Immunophenotypic examination using FACS-analysis revealed the co-expression of CD4/CD56 and raised the suspicion of BPDCN. The immunohistochemical stains of bone marrow confirmed the diagnosis and showed an infiltration grade of 90%. The patient had cutaneous manifestation at back as well. Since this neoplasm is derived from the precursors of plasmacytoid dendritic cells a therapeutic trial using bortezomib was performed in one patient with isolated cutaneous manifestation, but without success. Reviewing the literature BPDC neoplasms were successfully treated using intensive acute leukemia polychemotherapy followed by allogenic stem cell transplantation. Due to high toxicity rates associated with acute lymphoblastic leukemia-like regimes we decided to treat with intensive chemotherapy according to AML-like protocol. All three patients received induction chemotherapy with cytarabine (100 mg/m^2 on day 1-7) and daunorubicin (60 mg/m^2 on day 3-5). The skin lesions regressed clearly after one cycle of chemotherapy, and after the second cycle the cutaneous lesions had resolved completely. Bone marrow transplantation was carried out in one patient with isolated cutaneous manifestations. One year after allogenic stem cell transplantation the patient is still in complete remission and good health. The other two patients are waiting for allogenic stem cell transplantation. The patient with bone marrow involvement developed severe prolonged pancytopenia after induction therapy, which may be a result of bone marrow infiltration. Unfortunately the patient developed an invasive pulmonary aspergillosis. **Conclusions.** Blastic plasmacytoid dendritic cell neoplasm is a rare malignancy with poor prognosis. In our case report, AML-like induction therapy followed by allogenic stem cell transplantation represents an accepted treatment option with lower toxicity. However bone marrow involvement may be accompanied with prolonged pancytopenia and therefore high susceptibility to infections.

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PROGNOSTIC IMPACT OF LEUKOCYTOSIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MUTATED NPM1 AND FLT3-ITD

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Background. Mutation in nucleophosmin (NPM1) is the most frequently observed molecular abnormality, present in about 50% of acute myeloid leukemia (AML) cases, and is associated with a favorable outcome. Internal tandem duplication of the fms-related tyrosine kinase 3 gene (FLT3-ITD) is another frequent molecular abnormality and can be observed in about 25% of patients with AML. The presence of FLT3-ITD is generally considered as an unfavorable prognostic factor. **Aims.** The aim of this study was to investigate the prognostic impact of white blood cell (WBC) count at diagnosis on outcome within specific AML subgroups of patients with mutated NPM1 (as a favorable risk factor) and FLT3-ITD (as a poor risk factor). **Methods.** This single-center study involved 20 AML patients with mutated NPM1 and FLT3-ITD. The follow up period was 3 years. Patients with all other cytogenetic abnormalities and AML patients without cytogenetic abnormalities were excluded from this investigation. Patients were divided into three groups according to their WBC count: 1) a WBC count <20x10^9/L; 2) a WBC count 20-100x10^9/L and 3) a WBC count >100x10^9/L. Cox regression analysis was applied to determine the association of WBC and overall survival (OS) in months. **Results.** The mean age of the patients was 51 years (range 21-71). There was a strong correlation between the NPM1 mutation and a high WBC count, p = 0. 004. Also, differences in OS between the three groups of patients divided according to WBC count (<20x10^9/L vs 20-100x10^9/L vs >100x10^9/L) were statistically significant, p = 0. 003. **Conclusions.** This study demonstrated that leukocytosis

is an important unfavorable risk factor for OS in the subgroup of patients with AML and mutated NPM1 and FLT3-IDT.

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ARE WE CLOSE TO FIND CLEAR PROGNOSTIC FACTORS FOR SURVIVAL OF PATIENTS WITH ISOLATED MYELOID SARCOMA?

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Background. Isolated myeloid sarcoma (MS) is a extramedullary tumor of immature myeloid cells defined by the absence of a history of leukemia, myelodisplastic syndrom (MDS) or myeloproliferative neoplasm with a negative bone marrow biopsy. MS is very rare condition and has been described in limited case reports. Therefore, knowledge about the exact incidence and treatment of isolated MS is not clear but untreated isolated MS will almost always progress to acute myeloid leukemia (AML). The median time to the development of AML in this setting is about 5 months. Aims. We reviewed a single centre one decade experience and assess multiple prognostic factors with intention to determine the possible prognostic factors of survival outcomes in patients with MS. **Methods.** Eleven patients who underwent treatment for MS at Clinic for Hematology, Clinical Center of Serbia in period of 10 years (2002. to 2012.) were identified. Using Kaplan-Meier and Cox regression survival analysis we evaluated different prognostic factors: gender, age, MS localisation, CD68 expression, treatment options (chemotherapy (CT), surgery as well as auto/alo hematopoetic stem cell transplantation (HCT)) as possible prognostic factors that determining survival in patients with MS. **Results.** In our group consisted predominantly of males (7m/4f) the mean patient age was 37. 3 years (range, 19-68). Affected sites were: lymphoid tissue (3 patients), female genital tract (2 patients), testicular tissue, spine, spleen, gastrointestinal tract, bone, hearth. Patients were treated with chemotherapy (n=6) or surgery (n=5) combined or not with adjuvant radiotherapy. In 3 patients HCT (2 alo, 1 auto) was performed. After CT a total of 3 patients (50%) achieved complete remission and one patient achieved partial remission. During follow up period 8 patients died. The Kaplan-Meier estimates of median overall survival was 14 months (range, 2-36) while event (AML development or extramedulary relapse) free survival was 8 months. The patients undergoing chemotherapy had a overall survival of 14 months while patients treated with surgery alone had a overall survival of 13 months (p>0,05). Also, patients on CT had significantly longer event free survival time compared to those who did not (18 months vs 4 months, respectively, Log Rank=7. 484, p = 0. 006). Cox regression analysis identified a significant association between chemotherapy treatment and event free survival (p=0,029; RR=0,089; 95%CI for RR=0,010-0,784). Other investigated prognostic factors did not outperform the survival model, while the age bellow 40 remained significant prognostic factors in combination with chemotherapy. **Conclusions.** Based on our study population, MS presence implies rather a systemic instead of a localized aproach with surgery or radiotherapy. Early diagnosis and start of chemotherapy as soon as diagnosis is established can help in disease control and may promote a longer progression free survival time in patients with MS. Given the rarity of MS, collaboration of multiple cooperative groups to create a registry, tissue bank, conduct uniform correlative laboratory studies, develop clinical trials, and generate treatment guidelines is required.

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ACUTE MYELOID LEUKEMIA (AML) IN ELDERLY PACIENTS. ANALYSIS OF 92 CASES

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Introduction. Median age of patients with AML is 65 years and its incidence increases with age. Advanced age and associated morbidity hamper the selection of older patients to treat with intensive chemotherapy (QTi). Therefore, QTi is only offered to 30% of them. Results in terms of complete remission (CR), mortality and survival are worse than in younger patients. Poorer outcome and higher incidence of morbidities is associated with increased expression of the multidrug resistance gene, increased incidence of poor prognosis chromosome abnormalities and worse tolerance to QTi in this group. **Patients.** We describe 92 patients, 52 men and 40 women, all over 65 years, diagnosed with AML at our institution between June 2006 and Octuber 2011. M3 subtype was excluded. Median age was 76 years (63-95). 58. 6 % (54/92) were dismissed for QTi treatment due to advanced age and /or associated comorbidity. 41,3% (38/92) were selected for treatment with QTi. 17/38 (36%) had evolved from prior MDS. 8/38 (21%) had a complex karyotype at diagnosis. Induction chemotherapy was administered according to various regimens with the intention of completing 3 cycles in those who had achieved CR: IA (3X7): 5, attenuated IA (3x7): 20, IdaFLAG/FLAG: 9, Lenalidomide: 1 (with isolated 5q-), and 5-Azacitidine: 3. **Results.** Median overall survival (OS) of the total group was 5. 7 months. The OS of the patients treated with support therapy was 2. 5 months with a median hospital stay of 14 days, while the OS of the treated patients was 14. 9 months (4. 1- 25. 7), with a median hospital stay of 74 days. 55% (21/38) of patients undergoing QTi were resistant to treatment and all died. Of the remaining patients, 45% (17/38) achieved CR and all completed 3 cycles of treatment. Of these, 80% had been diagnosed of de novo AML and 20% had progressed from prior MDS. Only 1 of the 8 patients with complex karyotype achieved CR. The treatment-related mortality was 1/38 (3. 8%): one death in CR by aspergillosis. 13 of 16 patients (81%) relapsed and showed a median disease-free survival (DFS) of 18. 4 months (16- 20). 9 out of these 13 (58%) were rescued: 6 with QTi and 3 with 5-azacitidine. At the moment, 5/9 are still alive (all rescued with QTi). **Conclusions.** QTi induction treatment in elderly patients with AML prolongs survival in 11 months compared with supportive treatment and an acceptable median hospitalization stay. An adequate selection of patients for QTi treatment leads to a low mortality rate. The observed rate of resistance to QTi is similar to that described in the literature. Better responses are usually seen in patients with de novo AML and without poor prognosis cytogenetic.

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EVALUATION OF A PROSPECTIVE PROTOCOL OF THERAPY OF CHILDHOODACUTE MYELOID LEUKEMIA: A TUNISIAN MONOCENTRIC STUDY

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Background. Despite major achievements in the treatment of acute myeloid leukemia (AML) long term survival remains poor. About half of pediatric AML patients relapse and die. **Aims.** to study the outcome in children and adolescents treated with a local prospective protocol. **Methods.** Between January 2005 and December 2010, we analyze a prospective study, including patients, aged less than 20 years, diagnosed with AML, according to the WHO classification at department of hematology of Hedi Chaker hospital, Sfax. Secondary leukemia and FAB M3 were excluded. All patients treated with a protocol, contained an induction therapy: Ara-C (200mg/m^2/j on days 1-7) and Daunorubicine (60mg/m^2/j on days 1-3), if patients achieving complete remission (CR), consolidation therapy: 3 courses of consolidation chemotherapy (2courses with high dose cytarabine 18g/m^2/course+ Mitoxantrone or Asparaginase and 1 course withAraC200mg/m^2/j+Daunorubicine+Etoposide) or allogenic stem cell transplantation in presence of an HLA-identical family donor. We evaluated complete remission rate, toxic death induction, overall survival (OS) and event-free survival (EFS). The Kaplan-Meier method was used to estimate OS and EFS. **Results.** Twenty eight patients (15 girls and 13 boys) enrolled in our study. Mean age was 14. 3 years. WBC was >100000/mm^3 in 28%. According to the Medical Research Council's Trial (MRC): 5 patients were classified into favorable, 16 into intermediate group and 5 into unfavorable. Twenty patients (71%) achieved CR after one course. Five patients (18%) died during the induction therapy. Induction failure was observed in 3 patients (11%). Fifteen patients had a matched sibling donors but bone marrow transplantion was performed only in 4 patients. Relapse occurred in 6 patients (30%) in median of period 12 months. The overall survival and EFS are 44% and 40% at 5 years respectively. AML presenting with hyperleucocyosis than 100000/mm3 has been associated with high induction morality and had poor outcome. **Conclusions.** Despite significant improvement in outcome in pediatric acute myeloid leukemia in our

center, results of this protocol are still unsatisfactory. We need to improve them more by reducing induction mortality and more widely performed stem cell transplantation for patients achieving complete remission.

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DE NOVO ACUTE MYELOID LEUKEMIA (AML) IN ELDERLY AND EFFECTIVENESS OF INDUCTION THERAPY (IR)

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Background. Complete remission (CR) is associated with prolong relapse free survival of AML patients. There are different ways to improve the results of IR therapy. The problem of the second IR is not solved till now. **Aims.** To evaluate the response to the first and the second IR therapy and reveal the predictors of treatment outcomes. **Methods.** A retrospective analysis of the outcomes of 93 patients with de novo AML (excluding APL) was performed. The first course of IR was "7+3"scheme with Ara-C 100 mg/m^2 1-7 days and daunorubicin 60 mg/m^2 1-3 days. If there was no CR the same IR course or course with Ara-C ≥1 g/m^2 per injection ± antraciclyne was started. **Results.** The median age of patients was 48 y. (17-76). The results of the first IR were next: 55 patients with CR (59. 1%), 31 patients with no response (33. 3%) and 7 patients were died during first 28 days (7. 5%). The median age of patients with CR, no response and who were died was 43, 52 and 56 y.; *p=0. 097*. The rate of CR of patients with good, intermediate and poor ELN karyotype was 94. 4%, 55. 0% and 33. 3%, respectively; *r=0. 377, p=0. 0001*. There was no response to the first IR in 38. 3% and 53. 3% patients with intermediate and poor karyotype, respectively; *r=0. 344, p=0007*. The early death was fixed in 5. 6%, 6. 7% and 13. 3%, respectively; *r=0. 083; p=NS*. Among 26 patients with normal karyotype the rate of CR was higher if there was no *FLT3-ITD* mutation in comparison with the presence of *FLT3-ITD* independently of NPM1 status: 73. 7% vs 42. 9%; *p=NS*. The results of the second IR were evaluated in 26/31 patients with no response to the first IR. There were 19 patients with intermediate karyotype and 7 patients with poor karyotype. 16 patients were treated with scheme "7+3"and 10 patients with high dose Ara-C. There were only 3 CR (11. 5%). All patients with CR after second IR have intermediate ELN karyotype. **Conclusions.** Second IR "7+3"is not advisable to de novo AML patients with intermediate karyotype in the absence of response to the 1 standard IR. The possible condition to improve the results of IR in AML patients with poor karyotype is adding new drugs, e.g. purine analogue.

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TREATMENT OUTCOME IN YOUNG PATIENTS WITH ACUTE MYELOID LEUKAEMIA

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Background. Treatment of patients with acute myeloid leukaemia (AML) consists of an induction course followed by post remission consolidation. The post remission strategies for young patients are: intensive chemotherapy, autologeous stem cell transplantation, and allogenic haematopoietic cell transplantation. The choice of the optimal strategy is determined by a number of parameters including age, performance status, cytogenetic risk group and recently by molecular abnormalities. We report the results of a retrospective study in patients with AML treated in department of haematology of sfax (Tunisia). **Methods.** 60 patients are included from 2004 to 2009, aged between 20 and 55 years old. Patients with acute promyelocytic leukaemia or with histories of prior myelodysplasia are excluded. Cytologic diagnosis was performed according to the FAB classification. The chromosomal analysis was performed in all patients. The induction regimen was: Cytarabine 200mg/m2 continuous intravenous infusion for 7 days with Idarubicin 12mg/m2 (or Daunorubicin 45mg/m2 for 3 days). Consolidation strategy is based on cytogenetic classification, age and the obtaining of remission (CR) after one course, 3 groups are defined:Group1: favourable cytogenetic and CR after one induction (CR1)Group2: CR1 and intermediate cytogeneticGroup3: no CR1 and/or unfavourable cytogenetic* 3 cycles of high dose of cytarabine was proposed for patients included in group1* Patients bellow to group 2, 3 and patients bellow to group 1 and aged than 40 years old, received 3 courses of consolidation (ADE, MACE, and MIDAC)* An allogenic transplantation was proposed for patients < 40 years old with intermediate or unfavourable risk, when a HLA matched related donor is available. **Results.** The mean age was 36 years (20-55). FAB classification: M0 (4 %), M1 (20%), M2 (28%), M4 (18%), M5 (14%), M6 (3%), M7 (3%) and unclassified AML (10%) Karyotype: 16% low risk, 82% intermediate risk, 2 % high risk (MRC classification). The complete remission (CR) rate was 79 %, after one course it was 72 %. Death in induction was observed in 6 cases (10%). Primary failure was observed in 11 cases (18%). 36 patients received chemotherapy alone as consolidation. Only 11 patients undergo an allogenic transplantation in first remission. 4 deaths were noted in consolidation . The relapse rate was 33% (36% for patients treated with chemotherapy alone and 20% for who's received allogenic transplantation). Overall survival at 5 years was 44 %. **Conclusions.** We have better results than published in others local publications . However, it is required to improve the quality of hygiene and the supportive treatment to reduce mortality in CR, and performs allogenic hematopoietic cell transplantation to all AML patients with intermediate and unfavourable risk when possible.

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DEFERASIROX-ASSOCIATED SEVERE APLASTIC ANEMIA

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Background. Deferasirox is an iron chelating agent approved for the treatment of trasfusional iron overload. Pancytopenia is a well-known side effect mostly reported during post-marketing surveillance. True incidence of this complication remains unknown but all reported cases are mild to moderate. There have been no published cases of bone marrow aplasia to date. **Case Report.** We report the case of a 53 year old male with a medical history of myocardial infarction and unstable angina, as well as a locally advanced prostatic adenocarcinoma, treated with radiotherapy and androgen blockade, in remission at the time of hematology unit admittance. In June 2010 he was diagnosed with acute promyelocytic leukemia, carrying typical t(15;17) translocation. Patient was allocated to low risk group in Spanish Pethema LPA-2005 protocol, and therapy started. During induction therapy, several blood units were transfused in order to maintain a safe hemoglobin level and to avoid ischaemic angina. After consolidation therapy complete remission was assessed through bone marrow morphology and cytogenetics, both found to be normal, as well as a negative PCR detection for PML-RARalpha mRNA. Maintenance therapy was started in November 2010 with daily mercaptopurine and weekly methotrexate as well as intermittent ATRA. Doses were adjusted to maintain an absolute neutrophil count above 1500/µL, and by September 2011 doses of mercaptopurine (50 mg daily) and methotrexate (15 mg weekly) had been stable for several months. Iron status assessment showed a serum ferritin level of 2092 ng/mL; then iron chelating therapy with deferasirox was started at a dose of 500 mg once a day, aiming to a slow raise. In December 2011 bone marrow was obtained through aspiration and molecular absence of PML-RARalpha confirmed. Bone marrow cellularity was judged to be normal for patient age and no morphologic abnormalities were found. Blood platelet count was slightly decreased to 79,000/µL, but no dose adjustements in maintenance therapy were required according to Pethema LPA-2005 protocol. Deferasirox dose had been escalated to 1250 mg daily in January 2012, when severe pancytopenia was detected. The patient complained of petecchiae and bruising as well as severe asthenia and fever. Hemoglobin was 7,7 g/dL, leucocytes 300 with neutrophils 100/µL and platelets 5,000/µL. The patient was admitted to hospital for antibiotic therapy; etiologic studies for pancytopenia showed absence of hematopoietic cells, resulting in a diagnosis of severe bone marrow aplasia. Deferasirox, mercaptopurine and methotrexate were stopped, and pegylated GCSF administered as well as erythropoietin. Seven days after deferasirox withdrawal blood counts returned to normal, fever disappeared and the patient was fully recovered. Aplastic anemia was thought to be caused by deferasirox since no dosage variations had been made to mercaptopurine and methotrexate. These were soon reintroduced at former dosage with no substantial decrease in blood counts. **Conclusions.** clinical scenarios in which deferasirox is being used are increasing. As any new drug, further side effects and drug interactions might appear in the future. Post marketing surveillance essential to know actual risk associated with new drugs

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ACTIVATION STATE OF PLATELET IN EXPERIMENTAL SEVERE HEMOPHILIA A

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Hemophilia A (HA) is a rare X-linked hemorrhagic disorder consecutive to the absence of endogenous pro-coagulant FVIII. Abnormal FVIII levels are asso-

ciated with the development of crippling arthropathy and with elevated morbidity and risk of death. Depending on the residual activity of FVIII in plasma, HA is classified as severe, moderate or mild. Although this classification correlates with the bleeding phenotype, about 10% of severe HA patients rarely bleed spontaneously, indicating that FVIII activity is not the only parameter defining the phenotypic severity of the disease. Recently, van Bladel et al investigated the basal state of activation and responsiveness of circulating platelets as a potential parameter influencing the bleeding phenotype in patients with HA. They found that the state of activation of platelets is up-regulated in patients with severe HA, as compared to patients with mild/moderate HA and to healthy donors. An earlier report from Grünewald et al had however failed to find signs of enhanced platelet pre-activation in patients with severe HA. In order to address this question *in vivo* in a system that is not 'perturbed' by differences in genetic or environmental factors that are typical of the human population, we thus compared the states of activation and responsiveness of platelets from wild-type mice and from FVIII-deficient mice, an experimental model of severe HA. Our results indicate that the absence of pro-coagulant FVIII in un-manipulated adult animals is associated neither with alterations in the activation status of circulating platelets nor with their ability to be activated.

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HEMORRHAGIC DIATHESIS IN THREE PATIENTS WITH NORMAL HAEMOSTASIS TEST

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Background. Deficiency of F XIII is very infrecuent, it can cause spontaneous CNS haemorrhage in 30% of patients. The acquired form is very aggresive and may cause haemorrhages with mild reduction in factor levels; it appears frecuently in physiological stress circumstances (surgery, traumatism and disseminated intravascular coagulation) or by a non-antibody mediated drug-induced (statin, valproate) reduction. Is being the most frecuent underdiagnosed coagulopathy because the fact of normal routine test. We describe a serie of 3 cases of acquired deficit in our center. **Case 1.** 33-months oldchild with LAL-B under induction chemotherapy that 72hours later develops lowered level of consciousness and cardiorespiratory arrest after mild craneoencephalic trauma. Cardiopulmonary ressucitation and tracheal intubation were needed. Cranial CT scan: left subdural haematoma with active bleeding and secondary uncal herniation. Bleeding was controled with neurosurgical drainage. Clotting studies were requested because of minor head trauma. Haemostasis test: Fibrinogen: 5,1 g/L, Von Clauss Fibrinogen: 2,3 g/L, D-dimer: 1083 µg/L, Antithrombin III: 145%, Thrombin time: 23,9", Reptilase time: 22,7", XIII factor: *42,8%* with negative qualitative inhibition study. Treatment: 4 days administration of fresh frozen plasma (FFP) (10 mL/kg) to achieve levels of factor around 60%. Levels were normalized on 21th day of hospitalization and patient developed residual spastic tetraparesis but reached medular complete remission of LAL. **Case 2.** 77-years old man with aortoiliac aneurysm and left hypogastric artery embolization, admited at the hospital to aneurysm endoprothesic surgery. Torpid postoperative period with endoleak and incoercible inguinal haemorrhage requiring transfusion of 26 U of red blood cells and 1 platelet pool. Haemostasis test: Fibrinogen: 2,8 g/L, Von Clauss Fibrinogen: 1,6 g/L, D-dimer: 3476 µg/L, Antithrombin III: 81%, Thrombin time: 27,5", Reptilase time: 21", XIII factor: *42%*. Treatment: infusion of 9 FFP units, suspensión of prophylactic anticoagulation and antiaggregation therapy until bleeding control, suspension of statin and local compression. On 20th day of postoperative period levels of factor become at normal values with subsequent good progress. He was discharged on 78th day with a normal bypass functioning. **Case 3.** 72-years old man with recently right femoral pseudoaneurysm surgery, admited at the hospital to study a 12 cm nonpulsatile mass localized on surgery zone. He needed surgical drainage of haemathoma and developed torpid postoperative period requiring transfusion of 6 U of red blood cells and various antibiotic treatments because of wound infection. Haemostasis test: Fibrinogen:2,4 g/L, XI factor: 84,8%, XIII factor: *38,2%*. Treatment: infusion of 10 FFP units, iron therapy, recombinant human erythropoietin and tranexamic acid**.** Levels of factor reached normalization on day 15 after FFP infusion. **Conclusions.** the diagnosis of F XIII deficiency requires a high clinical suspicion and it must be considered in patients with recurrent bleeding in postoperative period as well as in patients receiving statin or valproate therapy in spite of normal standard coagulation tests. To define if routine preoperative F XIII activity screening is a cost-effective policy remains at present undefined requiring further research in this setting.

1248

CUTANEOUS HYPERSENSITIVITY REACTION FOLLOWING THE USE OF TWO RECOMBINANT FACTOR VIII PREPARATIONS, KOGENANTE® BAYER AND ADVATE®: A THERAPEUTIC CHALLENGE SOLVED BY FACTANE®

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Backgroud. A severe hemophilia A boy was started prophylaxis when he was 9. 5 months old using 250 UI twice a week of Kogenate® Bayer for cutaneous bleedings. Dose was rapidly increased to 500 UI twice a week. However, at 9 CED and 3500 IU of Kogenate® Bayer an inhibitor was detected at a level of 104 BU. He was then shifted to NovoSeven® on demand for 316 days until inhibitor level was 2. 5 BU. Immune tolerance induction was then started with Kogenate® Bayer at a dose of 93 IU/kg twice a day. After the 2nd infusion, he developed an urticaria allergic reaction. Despite the use of antihistaminic and prednisone, urticaria reaction was higher for the subsequent infusion. Methods. After discussions with the French hemophilia reference center it was decided to replace Kogenate® Bayer with Recombinate® at the same dose. We took the precaution to maintain antihistaminic and prednisone for the first infusion of Recombinate®. However, despite this preventative measure a very similar urticaria reaction was observed. Since these two recombinant factor VIII were from different cell lines (BHK and CHO) but both purified with monoclonal antibody affinity column, it was then decided to try to pursue the immune tolerance induction using a highly purified plasma derived factor VIII that was not using monoclonal antibody in its process. Factane® was then used at the same dose. **Results.** No cutaneous reaction was observed, and immune tolerance induction was fulfilled in 351 days with Factane®. **Conclusions.** With a follow up of two years, he is still with a normal recovery, a half life > 8 h and an inhibitor < 0. 6 BU under a well tolerated prophylaxis using Factane®.

1249

PSYCHOSOCIAL IMPLICATIONS OF THE DIAGNOSIS OF HEREDITARY THROMBOPHILIA

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Background. Thrombophilia is an inappropriate tendency to thrombus formation. Its causes are hereditary or acquired and often may be associated. In recent years numerous studies were conducted in the field of heritable and acquired thrombophilia, in an attempt to prevent the consequences of thrombotic disease. However, there are few studies to evaluate impact of diagnosis on quality of life and socio-economic implications of this situation. **Aims.** Our aim was to evaluate the psychological impact of the diagnosis of hereditary thrombophilia on the patients. **Methods.** Between 02. 2010-01. 2012 in Oncomed Timisoara we evaluated 104 patients with thrombophilia, 94 women and 10 men. A questionnaire was given to every patient, to assess the quality of life. **Results.** There was a major difference between men and women, all men had a normal life, without any thoughts about the disease. All of them were taking 75 mg of aspirin per day, without any other concomitant medication. Their emotional well-being score was the same with the score of general population. Between women, only 70 patients accepted to answer the questionnaire. This can be explained by the implication of thrombophilia diagnosis in women and the anxiety they felt about their disease and the failure to conceive. All non-pregnant women were taking 75 mg of aspirin (70) and all pregnant women were taking LMWH in different doses according to weight (24). Among pregnant women, emotional well-being score was not different than in general population, but was better than in women who did not achieve pregnancy. These women were having thoughts of hope- 70%, but 85% of them felt frustration and 27% - fatigue. Differences in QOL were observed by number of miscarriages, the patients with more than 2 miscarriages were more depressed than those with one miscarriage or none. Factors associated with lower quality of life included age, severity of co-morbid health conditions (other obstetrical conditions) and number of miscarriages and thrombophilia type (those with homozygous condition felt more depressed). None of them resigned or were expelled due to low quality of daily work**. Conclusions.** Thrombophilia has a profound impact on QOL of patients. This result shows that a close collaboration between hematologist, gynecologist and psychologist is the key to a good quality of life for these patients.

1250

DABIGATRAN INTERFERENCE IN FOLLOW-UP OF ANTI-THOMBIN EFFECT OF MONOCLONAL IMMUNOGLOBULIN

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Introduction. In case of monoclonal anti-globulin, haemostatic disorders as anti-thrombin (anti-IIa) effect can be observed. Haemorrhagic syndrome can happen. In other hand, dabigatran, new oral anticoagulant treatment, with reversible direct anti-IIa effect, is administrated without biological follow-up, prolonges usual coagulation time. In this case is reported an interference of dabigatran on haemostasis tests for checking anti-thrombin effect of a monoclonal immunoglobulin. **Observations.** A 74 year-old female followed for a IgG Kappa (28. 9 g/L) myeloma benefits from a preoperative assessment of haemostasis before a total knee prosthesis (TKN). The activated partial thromboplastin time (APTT) Patient (P)/Control (T) (48/30 seconds), the prothrombin time (PT) P/T (20/13 seconds), and the thrombin time (TT) P/T à 163/20 are prolonged. The fibrinogen dosage (von Claus method) is low (1. 2 g/L; 2. 5-4. 5 g/L) . A normal reptilase time with prolonged TT without unfractionated heparin points out a anti-thrombin effect of the monoclonal immunoglobulin. The patient has no sign of haemorrhagic diathesis and considering these results, the surgery is reported. A first remission of myeloma is achieved after 12 months of chemotherapy with Alkeran®, Cortancyl® et Velcade®. The APTT is then P/T 38/30 s, the PT is 14 s, TT P/T 74/20 s and fibrinogen 2. 4 g/L, with a stable electrophoretic peak of 15 g/L. Two months later, haemostasis tests show again a prolonged APTT à P/T 74/30 s, a prolonged TT >180 s and a normal TR. Anti-Xa activity is lower than 0. 1 UI/mL without increase of the electrophoretic monoclonal peak (14. 5 g/L). The thrombin generation assay shows a profile compatible with a direct anticoagulant anti-IIa. After discussion with physician in charge of the patient about the discordance of prolonged APTT and lack of increase of the electrophoretic monoclonal peak, the information was given that the TKN has been done without any complication and that the patient undergoes a prophylactic treatment by dabigatran after the surgery. **Conclusions.** This case report is an example of the possible difficulties of the interpretation when a new oral anticoagulant treatment is given which, because of the lack of need for laboratory monitoring may not be filled by prescribers.

1251

DESMOPRESIN TEST IN A CASE OF SEVERAL BLEEDING DISORDERS IN NOONAN SYNDROME: LITERATURE REVIEW AND A CASE REPORT

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Background. Noonan Syndrome (NS) is a common genetic disease with multiple organ defects. Bleeding diathesis is considered part of the clinical findings with a prevalence of 1/1,000 to 1/2,500. The syndrome was first described in 1963 by Noonan and Ehmke. The hematologic disorders associated with this syndrome were last reviewed in 2011 by Benjamin J. Briggs, and Joseph D. Dickerman. Four etiologies are postulated to explain the NS bleeding disorders: thrombocytopenia, platelet dysfunction, VWD, and factors deficiencies. NS often combine several bleeding disorders, therefore, if a patient has one bleeding disorder is expected to have another. A large number of patients with NS require surgery for congenital heart disease, cryptorchidism, lymphatic vessel anomalies or cosmetic reason. Therefore is clinically important a knowledge of bleeding disorders in NS. **Aims.** Provide a case to make it available for both pediatricians and hematologists professionals in their clinical practice since there is little literature about. MethodsA four years old patient diagnosed of NS and mild pulmonary valve stenosis was derived to coagulation unit from pediatric surgery for active bleeding started 48h after surgery of right cryptorchidism. **Results.** Coagulation study revealed an elongated APTT ratio of 47. 20 sec, the rest of results are shown below:VWF:Ag:62. 90%, VWF:RiCof: 48. 20%, FII:120. 70%, FV:105. 30%,FVII:94. 80%, FVIII:103. 40%,FIX:106. 3%,FXI:106. 80%,FX:122. 10%,FXI:106. 80%, FXII:79. 70%, FXIII:65. 50%, circulating antibody negative. A slight deficiency of VWF and FXIII was detected and we performed an intranasal DDAVP test. The results are summarized in Table 1. **Conclusions.** NS often combine several bleeding disorders, therefore, if a patient has one bleeding disorder is expected to have another as shown in this case. There are no standard treatments for bleeding complications in patients with NS and recommendations regarding management are derived from case studies. The perform of desmopressin test may be useful in patients with Von Willebrand mild factor deficiency and Noonan Syndrome to show its effectiveness as antihemorrhagic treatment in case of surgery or spontaneous bleeding. A hematological investigation, especially prior to an invasive procedure, is required with some frequency in this disorder to avoid bleeding complications.

Table 1.

DESMOPRESIN TEST				
	Basal	One hour after	Four hours after	Normal Laboratory values
VWF:Ag	46.40 (57-170)	92.80	95.60	(57-170)
VWF:RICof	39.70 (50-150)	96.20	77.30	(50-150)
F VIII %	96.10 (70-150)	379.60	227.40	(70-150)
PT %	95.70 (70-130)	90.40	83.50	(70-130)
aPTT sec	36.70 (25-38)	27.60	30	(25-38)
aPTT ratio	1.20 (0.80-1.20)	1.10	1.00	(0.80-1.20)
PFA ADP	117 (71-118)	217	97	(71-118)
PFA Norepinephrine	166 (94-193)	72	99	(94-193)

1252

SUCCESSFUL USE OF RECOMBINANT ACTIVATED FACTOR VII FOR A MAJOR SURGERY IN A PATIENT WITH SEVERE FXI DEFICIENCY AND SEVERE ALLERGIC REACTION TO FRESH FROZEN PLASMA

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Severe congenital FXI deficiency is the commonest of the rare bleeding disorders. In contrast to hemophilia A or B, bleeding tendency in FXI deficiency do not correlate with FXI level. Therefore, optimal management of the patients with FXI deficiency is difficult. In addition, the bleeding risk for a surgery depends upon the type and the site of surgery and increases in high fibrinolytic areas such as the mouth, and nose. Several therapies such as fresh frozen plasma (FFP), FXI concentrate, fibrin glue, antifibrinolytic drugs are available for these patients. In the last years, the successful use of recombinant activated factor VII (rFVIIa) has been reported in a very limited number of cases, including those with inhibitors. This is an off-label use of this product. The dose of treatment and the duration has varied greatly between these case reports. In this case report, we present our successful experience with the use of rFVIIa for repairing the alveolar cleft and anterior palatal fistula in a 7-year-old boy with severe congenital FXI deficiency and severe allergic reaction to FFP. The patient was born to first degree consanguineous parents with the clefts of lip and palate. He was diagnosed as a severe congenital FXI deficiency (FXI:C level 0. 2%) after the bleeding from the cleft lip repair surgery when he was 6 months old. First successful cleft palate surgery was done with FFP concentrates and tranexamic acid, when he was 18 months old baby. He was followed-up in our unit. The repairing surgery of alveolar cleft and anterior palatinal fistula was planned when he was 6. 5 years old. However, severe allergic reaction developed during FFP infusion at the dental extraction before surgery. For this reason, we planned to use of rFVIIa for his surgery. Tranexamic acid was started orally every eight hours the day before surgery, and was given intravenously two hours before surgery, and rFVIIa was given 40 µg/kg one hour before surgery. Secondary alveolar bone grafting technique was performed under general anesthesia for the patient who was in his period of mixed dentition. Ilium was chosen as the donor site for obtaining autogenous cancellous bone grafts. The second dose of rFVIIa was given 4 hours later after the first dose, and the dose of rFVIIa was reduced to 20 µg/kg, and continued at 6 hourly intervals for the first day, 8 hourly for the second day, 12 hourly for the 3rd and 4th day, and once a day for 5th and 6th day at the same dosage. Tranexamic acid was given until to 10 days after the surgery. No excessive bleeding or thrombosis were observed. In conclusion, low-dose rFVIIa therapy was successfully used for a major surgery in our patient with severe FXI deficiency and severe allergic reaction to FFP. However, treatment safety is a major concern and the best dosing regimen of this off-label drug is still to be defined for the selected patients.

1253

SPONTANEOUS RUPTURE OF SPLEEN IN A CHILD WITH EWAN'S SYNDROME AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Background. Spontaneous rupture of spleen is a rare but documented complication of hematologic, infectious and neoplastic diseases. Splenic involvement has been reported in collagen vascular diseases and vasculitis, but splenic rupture is extremely rare. Because of high mortality rate, early diagnosis and treatment are life-saving. In patients with systemic lupus erythematosus (SLE), the management of splenic rupture may be difficult because of thrombocytopenic conditions. Herein, we report a case of spontaneous splenic rupture which occurring background of SLE and Ewan's Syndrome. **Case Report.** A previously healthy 15 year-old boy was referred to our clinic because of pancytopenia. Physical examination revealed paleness, jaundice and mild splenomegaly, 1 cm below left costal margin. Laboratory results included white blood cell count of 2. 3x109/L, hemoglobin level of 6. 5g/dl, platelet count of 12x109/L. Normoblastemia, reticulocytosis, indirect hyperbilirubinemia and positive direct antiglobulin test which were showing autoimmune hemolytic anemia were detected. Urine analysis showed hematuria and proteinuria. Serum levels of kidney function tests (urea, creatinin) were in normal range. Bone marrow examination revealed that erythroid hyperplasia, increased number of early megakaryocytic cells, rare thrombocytes and no maling infiltration. Bilateral pleural effusion was seen on chest X-Ray. Antinuclear antibody and anti-double stranded DNA were positive. These findings were consistent with Ewan's syndrome which occurring background of SLE. Steroid treatment initiated to patient, but no clinic and hematologic response achieved. Cyclophosphamide was added to therapy on day 7. On the 10th day of hospitalization, he was clinically shocked with tachypnea, tachycardia and hypotension. Abdominal distension with tenderness of left upper quadrant was present on his abdominal examination. Spontaneous rupture of spleen and intraabdominal hemorrhage were detected by abdominal ultrasound and computerized tomography scan (Figure 1). There was no history of trauma. Simultaneously, complete blood count revealed that white blood cell count of 2. 6 x109/L, hemoglobin level of 5. 8g/dl, platelet count of 12x109/L. Prothrombin time (12 sec) and activated partial thromboplastin (28 sec) time were in normal range. Immediate fluid resuscitation was applied, he transfused with 2 units of packed red cells and 1 unit of platelet suspension prepared by method of apheresis because of life-threatening bleeding. Although the severe thrombocytopenia, intraabdominal hemorrhage were controlled successfully without any additional transfusion. The patient did not need to abdominal surgery, because the patient's hemoglobin levels did not decrease anymore. The patient's vital signs were gradually improved. In the following period, intraabdominal bleeding was resorbed at day14. Plasmapheresis was applied to patient when the patient's situation was convenient. The patient's cytopenias were improved at the end of one month of therapy. **Conclusions.** Our case indicates that splenic rupture may occur in the course of SLE even without massive splenomegaly. The control of bleeding may be difficult in the presence of Ewan's syndrome and thrombocytopenia. If a patient with non-traumatic splenic rupture, hemodinamically stable, conservative treatment may be preferred to avoid surgical complications. Serial ultrasound or computerized tomography scan are useful to monitoring of the resolution of the intraabdominal bleeding and the healing of the spleen.

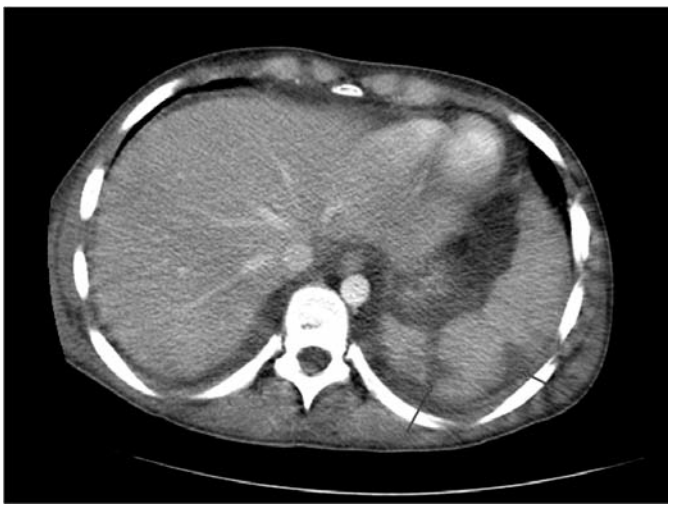

Figure 1. Axial CT image showing splenic rupture.

1254

ESTABLISHMENT OF KOREAN STANDARD FOR BLOOD COAGULATION FACTOR IX CONCENTRATE

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Background. National Standard for Factor IX had not been established yet in Korea and the working standard plasma material was used as replacement for the reference standard to measure factor IX potency. It has different factor IX content on every batch and there are other coagulant factors besides IX. **Aims.** A collaborative study among four laboratories including three manufacturers and one national control laboratory was carried out to evaluate the suitability of a candidate to serve as the 1st Korean Standard for blood coagulation factor IX. **Methods.** The candidate material for national standard was manufactured according to the WHO guideline for biological standards and the Minimum Requirements for Biological Products in Korea, issued by KFDA. It was distributed to four laboratories and assayed using a one-stage clotting assay with two kinds of equipments against the 4th International Standard for FIX Concentrate (07/182) to determine the potency of this candidate. Accelerated thermal degradation at -20, 4, 20, 37, 45℃ and the real-time stabilityuntil 18 months for long-term storage were examined. The 3rd IS for Blood Coagulation Factors II, VII, IC and X, Plasma, Human (99/826) and Standard Human Plasma was also included to evaluate the relationship between the factor IX plasma and concentrate unitage. **Results.** One hundred thirty-one sets of clotting assay results were analysed. Based on the data collected from all participants, intra-laboratory variability was found to range from 2 -5. 55% and good inter-laboratory agreement with the majority of GCV around 5% was obtained. Stability studies indicated that the candidate was very stable. The way to measure the clotting time depends on the equipment showed little differences on the potency value. The estimated mean value obtained from the one-stage clotting assay was 12 international units (IU)/vial. **Summary and Conclusions.** As the result of this collaborative study, the candidate standard is adopted as the 1st Korean National Standard for Blood coagulation factor IX, concentrate. It is expected to contribute the globalization of quality control in factor IX product by establishing the national standard.

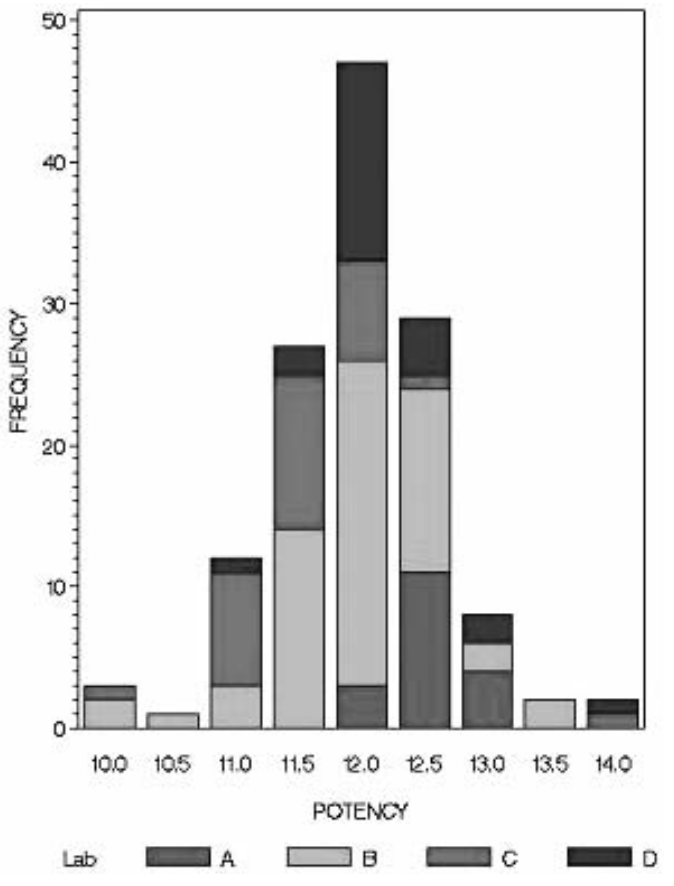

Figure 1. Histogram of potency estimates by laboratory.

1255

HEMATOLOGICAL MANIFESTATIONS OF NOONAN SYNDROME: A CLINICAL CASE

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Background. Noonan syndrome is a congenital disorder with multisystemic achievement that, in addition to cardiac involvement (congenital heart defects and heart disease), gastrointestinal, mental and growth retardation and dysmorphic facies, also affects platelets (number and function) and the coagulation system, with hemorrhagic manifestations described in the literature as easy bruising, epistaxis and menorragias. The bleeding can be significant during and after surgical procedures. **Aims.** Description and analysis of a clinical case, focusing on the clinical and hematological findings in a female teenager with Noonan syndrome waiting for cardiac surgery. **Methods.** Collection of clinical data contained in the medical record of the patient and review of recent literature. **Results.** The authors present the case of a 13 year-old teenager that had since newborn age a dismorfic syndrome associated to a left ventricular hypertrophic cardiomyopathy. Hematologically she presented a severe thrombocytopenia, moderate anemia without other cytopenias and slight increase of hemoglobin F. The diagnosis of Fanconi Anemia was excluded. The former diagnosis was Amegakaryocytic Thrombocytopenia face to the presence of no megakaryocytes in bone marrow. Paradoxically along the years the platelet count showed a progressive increase. At four years of age the diagnosis was reviewed to Hipomegakaryocytic Thrombocytopenia. The platelet count stabilized at 100000/mm^3, but with the presence of a moderated hemorrhagic syndrome. There is maintenance of a mild anemia, without leucopenia or neutropenia, and the levels of hemoglobin F became normal. At 12 years-old the diagnosis of Noonan Syndrome was considered and confirmed at molecular level. At this time hemostasis was reevaluated. This study showed an abnormal APF-100 test (Colagen/ADP>246,0 sec. and Colagen/Epinephrine>280,0 sec.) disproportionate to the value of thrombocytopenia, suggesting platelet dysfunction. This platelet dysfunction was confirmed by platelet aggregation studies. The assay of FVIII: C was normal. The von Willebrand factor (vWF) despite being quantitatively normal (vWF: Ag - 82. 5%) had impaired function (vWF: RCo - 43. 9%). This evaluation is currently of particular importance since the child is waiting for heart surgery. **Conclusions.** The authors highlight the need to evoke the Noonan Syndrome facing an unexplained thrombocytopenia, especially if associated with a dysmorphic syndrome and/or disproportionate hemorrhagic symptoms. They also emphasize the importance of a careful investigation in these patients, performing coagulation screening tests, especially before surgical procedures, often needed in this disorder. The hematologist can play a key role in appropiate evaluation and management

Table 1. Hemostasis tests results.

Test	Result	Reference values
Activated partial thromboplastin time (APTT)	34.2 sec.	27.9 sec.
	1.21 Ratio	
Prothrombin time (PT)	12.7 sec.	10.1 sec.
INR (International Normalized Ratio)	1.09	
FVIII: C	98.00 % of N	
FvW: Rco	43.9 % of N	
FvW: Ag	82.5 % of N	
Platelet Count	100000/µL	
APF100 Colagen/Adenosine Diphosphate (ADP)	> 246.0 sec.	56 a 120 sec.
APF100 Colagen/Epinephrine (Col/Epi)	> 280.0 sec.	106 a 146 sec.
Aggregation with colagen (2µg/mL)	Normal	
Aggregation with ADP (10µM)	Normal	
Aggregation with ADP (20µM)	Normal	
Aggregation with arachidonic acid (1mM)	Normal	
Liberation of ATP* (Agonist: Colagen)	Normal	
Liberation of ATP (Agonist: ADP)	Absent	
Liberation of ATP (Agonist: ADP 20µM)	Absent	
Liberation of ATP (Agonist: Arachidonic Acid)	Decreased	
Agglutination with ristocetin (1mg/mL)	Normal	
Agglutination with ristocetin (0.5mg/mL)	No Agglutination	

Note - Platelet count in platelet-rich plasma - 104000/µL
* Adenosine triphosphate

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SURGICAL INTERVENTIONS IN OUR HEMOPHILIA PATIENTS

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Surgical procedures in hemophilia patients may be performed safely as in healthy individuals by providing appropriate factor therapy and preperation. Abnormal tests of haemostasis prior to surgery may cause anxiety and hesitancy in surgical team. Inspiration of confidence to surgeons by hematologists may help overcoming this situation. 158 surgical prosedures performed in various surgical clinics, on 83 hemophilia patients followed up in our clinic between 1990-2011, were evaluated retrospectively. Of these patients 71 were hemophilia A (3 with inhibitor) and 12 were hemophilia B. The ages at the admission were between 2 months-18 years. Of these patients 51 had severe, 14 had moderate and 18 had mild hemophilia. Performed procedures include; r*adioisotope synovectomy (*54 patients), circumcision (50 patients), tooth extraction (23 patients), various orthopedic interventions (14 patients), drainage of in central nervous system hematomas (7 patients), appendectomy (3 patients) and other various procedures (7 patients). In 44 surgical procedures antifibrinolytic agents were also used in addition to factor replacement therapy. Six patients were diagnosed due to prolonged bleeding during or after surgical procedure. Postoperative late bleeding was encountered and treated in four patients. None of our patients died due to bleeding and antibody development was not observed in any patients. In our report, preparation for surgical procedures, factor replacement prior and after surgery, and postoperative complications in hemophilia patients are discussed.

1257

CONTINUOUS ACTIVE STATE OF COAGULATION SYSTEM IN PATIENTS WITH NONTHROMBOTIC INFLAMMATORY BOWEL DISEASE

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Aims. This study was planned for searching possible changes of the total coagulation and fibrinolysis system in inflammatory bowel disease (IBD) in order to obtain some clues for explaining the relation between IBD and hypercoagulability. **Methods.** A total of 24 patients with ulcerative colitis, 12 patients with Crohn disease, and 20 healthy controls were studied. Platelets; prothrombin time (PT); partial thromboplastin time (PTT); fibrinozgen; D-dimer; fibrinogen degradation products; protein C; protein S; antithrombin; thrombin time; von Willebrand factor; coagulation factors V, VII, VIII, IX, XI, and XIII; plasminogen; antiplasmin; tissue plasminogen activator; plasminogen activator inhibitor 1; and prothrombin fragments 1 + 2 were studied. **Results.** Most of the procoagulants (platelets, fibrinogen, von Willebrand factor, coagulation factor IX, and plasminogen activator inhibitor 1) were found increased together with decreases in some anticoagulants (protein S and antithrombin) in IBD. Also the activation markers of coagulation D-dimer, fibrinogen degradation products, and prothrombin fragments 1 + 2) were all increased. **Conclusions.** The parameters of the total coagulation-fibrinolysis system were increased in IBD, regardless of the form and the activity of the disease.

1258

SUCCESSFUL PROPHYLACTIC USE OF RECOMBINANT ACTIVATED FACTOR VII (RFVIIA) IN A PATIENT WITH CONGENITAL FVII DEFICIENCY AND INHIBITORS TO FVII

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Background. Congenital factor VII (FVII) deficiency is a rare, autosomal recessive bleeding disorder. The clinical phenotypes range from asymptomatic condition to severe disease characterized by life-threatening bleeds including intracranial hemorrhage (ICH) or gastrointestinal bleeds. **Case Report.** A 3-month-old boy presented with intracranial haemorrhage and was diagnosed

with severe congenital FVII deficiency. Genotyping identified a homozygous, nonsense mutation (p. Ser112Stop) in the FVII gene. Using x-MAP® technology, we showed that the patient developed inhibitors against rFVIIa with high antigenic reactivity, with a progressive switch to IgG4 subclass during the follow-up treatments. The bleeding episodes were successfully treated with classical doses of rFVIIa, suggesting that such treatment saturates the inhibitory activity of the antibody. Despite the persistence of inhibitors, rFVIIa prophylaxis was successful and the patient reported nearly no bleeds for 2 years. **Conclusions.** Prophylaxis using rFVIIa should be recommended even for patients with inhibitors to FVII.

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ACQUIRED HEMOPHILIA: REPORT OF FIVE CASES. A SINGLE CENTER EXPERIENCE

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Background. Acquired hemophilia A (AHA) is an autoimmune disease caused by an autoantibody to factor VIII (FVIII) and is associated with various underlying conditions such as pregnancy, malignancy, autoimmune diseases, and allergic reactions to medications (penicillin, chloramphenicol, phenytoin, etc.). However, in almost 50% of cases not determined a causal factor. It has a high morbidity and mortality and the incidence is estimated about 1 case per million populations per year. Mortality rate reported is between 10 - 22% and is due to severe bleeding complications or side effects of immunosuppressive therapy. Perhaps the ignorance of the disease by health professionals, contribute to this incidence has been underestimated. Prognosis of this disease depends of an early diagnosis and therapy. The literature available on this subject comes from case series and retrospective studies. The largest cohort reported to date is the European Acquired Hemophilia Registry (EACH2) with data of 501 patients prospectively reported. It was created with the objective of solving unknowns in the management of clinical bleeding and inhibitor eradication, but these data are only available in abstract. **Aims.** We describe a series of 5 patients with an AHA seen in a five years period in a university hospital in the south of Spain. **Methods.** We reviewed clinical data from patients who were classified with an AHA in the period from January 2007 to December 2011 at the Virgen de las Nieves Hospital, in Granada, in the south of Spain. **Results.** In this period 5 cases met the diagnosis of AHA, the average age was 33 years (range 29 to 80) with a male-female ratio of 1:4. Two of the 5 patients had an AHA in late postpartum, one patient was personal history of Evans syndrome, one of breast cancer and one of bilateral inguinal hernia. The symptoms were bruising and prolongation of APTT in all the patients, except in one of them, which presented active bleeding in the immediate postsurgical period. The average time until remission was 2,5 for months(range 1 to 7). The inhibitors were completely resolved in all patients only one of the five patients died due to opportunistic infection secondary to immunosuppressive therapy. Characteristics of the patients are shown in Table 1. **Conclusions.** Experience in our hospital with the usual treatment is satisfactory, with only a death due to infection secondary to immunosuppressive therapy in an older patient. The limited number of cases of AHA make difficult to establish their clinical course and to perform randomized trials. Therefore the management of patients with this disease is complicated because the literature available on this subject comes from case series and retrospective studies. Knowledge of the symptoms of this pathology by health professionals is critical because the prognosis depends on early diagnosis and treatment.

Table 1.

No	Sex	Age	Predisposing condition	Hemostatic treatment	Immuno-modulatory therapy	Time until complete remission	Outcome
1	F	33	Late Postpartum	Recombinant factor VIIa	Streroids+ cyclophosphamide+ Rituximab	One month	Favourable
2	F	29	Late Postpartum	Tranexamic acid	Steroids+Rituximab	Seven month	Favourable
3	F	30	Evans Syndrome	no	Steroids	One month	Favourable
4	F	80	Breast cancer	Recombinant factor VIIa	Streroids+ cyclophosphamide+ Rituximab	Four month	Favourable
5	M	74	Postsurgical	Recombinant factor VIIa	Steroids+ cyclophosphamide	No complete remission	Died

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IMPROVED CYTOTOXIC T CELL RESPONSES AGAINST A HETEROCLITIC HLA-A*0201 BINDING PEPTIDE DERIVED FROM THE 37/67 KDA LAMININ RECEPTOR PROTEIN

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Background. The 37/67kDa laminin receptor protein (37/67 kDa LRP) is a target molecule for immunotherapeutic studies in several tumor entities including hematologic malignancies. **Aims.** To identify a new heteroclitic-modified HLA-A*0201-presented peptide epitope (iLR-2het) deduced from the 37/67 kDa LR protein capable of eliciting tumor-specific human cytotoxic T cell (CTL) responses. **Methods.** T2-binding assays were combined with ELISPOT- and cytotoxicity assays. **Results.** Spontaneous T cell reactivity against the iLR-2het peptide was detectable in a significant proportion of patients with chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) compared to the native peptide iLR-2. In addition, iLR-2het-specific CTLs recognized and killed LRP-expressing CLL cells in an MHC-restricted manner. **Conclusions.** The identification of a new LRP-derived peptide epitope with high capability of eliciting CTL responses independent of the low binding ability of the naturally processed peptide increases the number of potential targets for peptide vaccination strategy against LRP expressing tumors.

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DEVELOPMENT OF ANTIBODY RESPONSES AGAINST HISTOCOMPATIBILITY-Y ANTIGENS AND THEIR X-VARIANTS AFTER ALLOGENEIC HEMATOPOEIETIC STEM CELL TRANSPLANTATION AND DONOR LYMPHOCYTE INFUSION

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Background. Allogeneic hematopoietic stem cell transplantation (alloSCT) is an effective treatment for patients with hematological malignancies. The beneficial graft-versus-leukemia (GvL) effect, however, is often accompanied with undesired graft-versus-host disease (GvHD). To reduce the incidence and severity of GvHD, T-cells can be depleted from the graft and readministered later as donor lymphocyte infusions (DLI) to preserve GvL reactivity. Evidence is accumulating that not only T-cells but also antibody responses coincide with GvL and GvHD. Antigens encoded by the Y-chromosome (histocompatibility-Y or H-Y antigens) have been described as targets for T-cells and antibodies in male patients transplanted with female donors, and immune responses against H-Y antigens are reported to correlate with clinical outcome. **Aims.** The aim of the study is to investigate the occurrence, specificity and biological relevance of antibodies against H-Y antigens during GvL and GvHD after treatment with alloSCT and DLI. **Methods.** Overlapping protein fragments for H-Y antigens ZFY and DBY and their respective X-variants (ZFX and DBX) were produced in E. Coli and coupled to fluorescent beads. In a high throughput Luminex bead assay, 1300 serum/plasma samples collected from 93 patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) before and during treatment with alloSCT and DLI were screened for antibody binding to the antigen-coupled beads. All sex-matched and mismatched patient-donor combinations were represented in the cohort (19 female-to-male, 17 female-to-female, 19 male-to-female and 31 male-to-male combinations). Mean fluorescence signals were normalized for background values as defined by reactivity against an irrelevant protein (HCV-core24). High titer antibodies were defined as $p<0.0001$ compared to the mean of all measurements for a given protein fragment. **Results.** Pre-existing H-Y reactive antibodies were measured in one male patient. Six of 93 patients developed H-Y antibodies after treatment with alloSCT and DLI. Five of these patients were male patients transplanted with female donors who developed antibodies against DBY (2x) or ZFY (3x) after treatment with DLI. In all 5 patients, antibodies against H-Y antigens were accompanied with reactivity against the respective X-variants, albeit in some cases with a lower fluorescence signal. Furthermore, in three of the five patients, detection of antibodies against H-Y antigens was associated with GvL reactivity and development of severe GvHD (grade II-IV, requiring systemic immunosuppressive treatment) . Of the remaining two patients, one patient displayed GvL reactivity without GvHD, whereas the other failed to develop any clinical response in spite of two administrations of DLI combined with intensive chemotherapy. **Summary and Conclusions.** We successfully used a Luminex bead assay for measurement of antibodies against multiple protein targets in a small sample volume and demonstrated that two ubiquitously expressed intracellular H-Y antigens are targets for antibodies in male patients transplanted with female donors. In these patients, humoral responses against H-Y anti-

gens and their X encoded variants were measured shortly after DLI and often coincided with development of GvL and severe GvHD. In conclusion, our data suggest that antibodies against H-Y antigens develop as a result of cellular debris induced by treatment with DLI, and that these antibodies display cross-reactivity towards X-encoded protein homologues.

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MATURE DENDRITIC CELLS CAN DIRECTLY INHIBIT THE GROWTH OF TUMOR CELLS IN VITRO

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Background. Dendritic cells (DC) are important antigen-presenting cells that involved in an induction of cellular and humoral immune response. This function is related to their ability to capture an antigen, express the immunostimulatory molecules and to migrate into lymph nodes. PRAME, cancer-testis antigen was chosen for DC pulsing because it is highly expressed in patients with hematogical malignancies and some solid tumors. Autologous dendritic cell vaccination is a promising strategy for adjuvant cancer therapies since DC are able to kill residual leukemic cells and/or prevent leukemia relapse. From recent studies we know that certain DC subsets have direct tumoricidal property by delivering cell death signals to malignant cells. **Aims.** To estimate the suppressing action of DC on the tumor cells by themselves in the culture in vitro. **Methods.** Immature leukemic dendritic cells have been cultured from mononuclear cells from bone marrow patient with acute myelogenous leukemia (AML) and from healthy donor blood, in the presence of GM-CSF, interleukin (IL)-4; mature DC were obtained in the presence of tumor necrosis factor (TNF)- and prostaglandin E. Part of DC was pulsed with recombinant PRAME antigen. DC and several human tumor cell lines (K562, THP-1, NOMO-1, U937) were co-incubated at different ratios. The viability of tumor cells was estimated by H3-thymidine incorporation assay as well as by trypan blue staining in the 3rd and 7th day of incubation. **Results.** We revealed that K562 cell line with high proliferation rate was more resistant to suppressive action of DC of healthy donor. Also we found that suppressive action of DC is reduced or disappear in condition of increasing of tumor cell growth rate. It is necessary that the number of DC considerably exceeds the number of tumor cells. In addition, specific loading DC with PRAME protein and consequent maturation significantly increases their initial tumor-suppressive activity. During co-incubation of mature DC from AML patient and K562 cells at a ratio of 1:1 the number of K562 cells decreased by 2,5 time; increasing the ratio of mature DC to 3:1 led to a reduction in the number of K562 cells to 7th day of incubation by 8 times. Thus the suppressive effect of DC from AML patient remains. **Summary.** It is shown that DC of donor directly cause the suppression of tumor cell growth and the effect of DC depends on the maturity of the DC as well as the rate of growth of tumor cells. So for developing of DC vaccine is necessary to control the conditions of maturing DCs, the number of input cells and load them to the appropriate antigen.

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HIGH EXPRESSION OF AURKA AND AURKB IS ASSOCIATED WITH HIGH WBC COUNTS AND COMPLEX KARYOTYPE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Aurora kinases are mitotic kinases especially important in the regulation of G2/M phase of the cell cycle and various mitotic events, including centrosomal duplication, mitotic spindle assembly, chromosome segregation and cytokinesis. The three major aurora kinases (*AURKA, AURKB* and *AURKC*) have been previously described and they are related to different stages of mitosis. Furthermore, aurora kinase overexpression leads to genetic instability and trigger the development of tumors. In fact, altered expression of aurora kinase proteins has been implicated in the pathogenesis of various cancers. **Aims.** In the present investigation, we have evaluated *AURKA* and *AURKB* gene expression in peripheral blood (PB) cells from chronic lymphocytic leukemia (CLL) patients and correlated these findings with hematological parameters and classical cytogenetics. **Methods.** Sixty two CLL patients (24 female and 35 male) and 10 age-matched healthy donors were selected. The comparative cycle threshold (Ct) method was used to evaluate expression of *AURKA* and *AURKB* genes in leukemic and normal samples. Metaphase induction in CLL was performed by using the immunostimulatory method that employs the combination of DSP30 and IL-2. Chromosome preparations were obtained by using standard procedures and the subsequent cytogenetic analysis and interpretation were made according to the ISCN (2009). All cytogenetic and gene expression data were validated by FISH analysis by using a specific set probes. According to median value of *AURKA* and *AURKB* gene expression, patients were divided into two groups (≥3. 4, considered as AURKA+ and >2. 3, considered as AURKB+) and their clinical and laboratorial characteristics were correlated. **Results.** Higher *AURKA* and *AURKB* expression were observed in CLL samples compared with normal samples (*AURKA* [mean value of ΔCt ± SD]: 9. 3 ± 3 *vs* 7. 1 ± 1, *p*=0. 02; *AURKB*: 16. 6 ± 1 *vs* 9 ± 2, *p*=0. 02). Moreover, *AURK*+ patients presented a significantly high leukocyte count compared with *AUR*- patients (*AURKA* [WBC count x 10^3 ± SD]: 37. 8 ± 5. 5 *vs* 68. 8 ± 5. 8, *p*<0. 001; *AURKB*: 40 ± 5. 5 *vs* 66. 6 ± 6. 2, *p*=0. 002, respectively). No significant differences were found regarding to Binet classification, gender or platelets count. Among the classical cytogenetic profile obtained, normal karyotype was found in 15 patients (24%) and metaphases with abnormal karyotype were seen in 47 subjects (76%). Interestingly, Pearson correlation showed a significant association between high expression of *AURKA* and *AURKB* and complex karyotype (relative risk: 2. 4 [95%CI: 1. 46-3. 93], *p*<0. 001). We demonstrated a significant correlation among high expression levels of *AURKA* and *AURKB* genes in CLL with chromosomal abnormalities and other hematological parameters. Overexpression of aurora kinase genes have been extensively studied in solid tumors. **Conclusions.** In CLL, our results may be associated to the genesis of chromosomal abnormalities and possible be used to predict the course of genomic instability in CLL patients.

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THE POTENTIAL ROLE OF UGT1A1*28 POLYMORPHISM IN THE PATHOGENESIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. It is assumed that Chronic Lymphocytic Leukemia (CLL) may be caused by the interindividual differences in the disposal of toxic substances resulting from polymorphisms in detoxification genes. UDP-glucuronosyltransferases (UGTs), which are Phase II conjugating enzymes, are involved in the detoxification of certain environmental toxins and carcinogens related to CLL. Studies in UGT1A1, a highly polymorphic gene and one of the most important UGTs, shed light to a possible cytoprotective role in human lymphocytes. UGT1A1*28 genetic polymorphism is characterized by an additional TA repeat in the $A(TA)_nTAA$ box region of UGT1A1 gene promoter. The dominant allele contains 6 TA repeats [$A(TA)_6TAA$], whereas the variant has 7 [$A(TA)_7TAA$] resulting in significant down regulation of UGT1A1 activity. **Aims.** We present a case-control study to investigate the potential risk effect of UGT1A1*28, an inborn polymorphism which has never been associated with any types of leukemia before, in CLL or CLL specific abnormalities. **Methods.** UGT1A1*28 polymorphism was investigated in 109 Greek CLL patients and 108 matched healthy controls using Real Time PCR (RT-PCR) assay. Fluorescence in situ hybridization (FISH) studies were performed on 64 patients to identify the commonest aberrations of CLL; deletions of 13q14. 3, 13q34. 3, 17p13. 1(p53), 11q22. 3(ATM) and trisomy 12 (+12). Statistical analysis was performed using Chi-square test and *P*<0. 05 was considered to be statistically significant. **Results.** The distribution of UGT1A1*28 polymorphism in CLL patients vs controls was: homozygous wild type (TA_6/TA_6) 39. 5% vs 39. 8%, heterozygotes (TA_6/TA_7) 40. 4% vs 45. 4%, homozygous mutant (TA_7/TA_7) 20. 2% vs 14. 8%. FISH analysis was successful in all 64 patients and cytogenetic abnormalities were detected in 43/64 (67. 2%). The comparative study of genotype and allele frequencies between CLL samples and controls revealed a significant high frequency of UGT1A1*28 in patients carrying abnormal FISH patterns (*P*=0. 020). Moreover, the incidence of TA_7/TA_7 genotype was higher in patients carrying +12 (*P*=0. 000) and TA_7 allele was found more often in patients carrying del(11)(q22. 3) (*P*=0. 016). However, no differences were observed in patients with del(17)(p13. 1), del(13)(q34. 3) and del(13)(q14. 3) (*P*>0. 050). Interestingly, TA_7 allele frequency was extremely high in patients with del(13)(q14. 3) followed by ≥1 aberrations (*P*=0. 000). It is noteworthy that all patients with del(13)(q14. 3) and +12 (n=4) carried the variant allele (25% TA_6/TA_7, 75% TA_7/TA_7). **Summary and Conclusions.** To the best of our knowledge, this is

the first study to investigate the correlation between UGT1A1*28 polymorphism and CLL. The distribution of UGT1A1*28 polymorphism indicated an increasing tendency in CLL patients homozygous for the mutant TA_7 allele vs controls. The high incidence of mutant genotypes found in patients carrying the commonest aberrations of CLL[+12,del(11)(q22. 3),del(13)(q14. 3)] suggests that UGT-deficient individuals may be at a greater risk for developing CLL specific abnormalities. Therefore, UGT1A1*28 polymorphism might be a predisposing factor to the pathogenesis of CLL. The chronic accumulation of toxic substrates implicated in CLL aetiology, caused by the lower corresponding catalytic ability of UGT1A1*28, supports the idea of a complex CLL biology, reliant on the interplay of inherited, environmental and host factors.

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T-LGLL AND NK-CLPD: TWO DISORDERS WITH A COMMON ETIOPATHOGENETIC MECHANISM?

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Background. The large granular lymphocytes (LGL) disorders are characterized by expansions of lymphocytes with cytotoxic activity. This expansion could be sustained by T or NK cells and WHO classification considers these disorders as separate entities, referred to T-LGL leukemia (T-LGLL) or NK-chronic lymphocyte proliferation disease (NK-CLPD). The marker of clonality is represented by the rearrangement of TCR in T-LGLL whereas a restricted pattern of Killer Immunoglobulin-like Receptor (KIR) expression is a characteristic figure in NK-CLPD. The etiology of LGL leukemia is largely unknown but several reports support the hypothesis of an antigenic stimulation as initial step, that would led to the expansion of LGLs which is not eliminated as a consequence of an impairment of apoptotic pathways and of a persistent inflammatory stimulation mediated by cytokines. Although these disorders are characterized by the expansion of different cells types, with specific genetic features and abnormalities, compelling evidences support the hypothesis that a common pathogenetic mechanism would be involved in both these disorders. **Aims.** We evaluated whether clonal T cell populations were detectable in patients with KIR restricted NK-CLPD, to corroborate the hypothesis that a persistent antigenic pressure takes place in these patients. **Methods.** We enrolled 50 therapy-free patients with NK-CLPD. All enrolled patients provided written informed consent in accordance with the Declaration of Helsinki. TCR gamma chain rearrangement was investigated by PCR in DNA purified from PBMC of NK-CLPD patients. KIR restriction was analyzed in all patients. **Results.** We found that in 27 out of 50 NK-CLPD patients (54%) a clearly detectable clonal T cell population was present, despite to the fact that all patients presented a NK disorder with a restricted pattern of KIR expression. The clonal T cell population ranged from a fairly detectable band to a strong signal. This surprisingly high percentage of T clones present in NK cell proliferation can not be explained by concomitant infections, since all patients were asymptomatic. Follow up analysis confirmed that TCR rearrangements were stable during time. Furthermore, in two patients we could demonstrate a switch between KIR restricted NK-CLPD to a monoclonal T-LGLL during a mean follow up of 5 years. **Conclusions.** Results of this study support the hypothesis that an antigenic pressure is taking place in patient with NK-CLPD. The demonstration of switch between NK-CLPD and T-LGLL further suggests that a common mechanism is involved in the pathogenesis of these disorders.

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CD38 GENE POLYMORPHISM AND RISK OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Objectives. Recent studies suggested the importance of CD38 signaling in the pathogenesis of B-cell chronic lymphocytic leukemia (B-CLL) and impact of rs6449182 *CD38* polymorphism on clinical course of disease. An association between genotypes rs6449182 of *CD38* and susceptibility to CLL was found in Polish Caucasians (Jamroziak et al., 2009) but these data were not confirmed in group of Italian CLL patients (Aydin et al., 2008). The aim of this paper was to study a risk CLL depending on rs6449182 of *CD38* in Ukrainian CLL cohort. **Patients and Methods.** rs6449182 CD38 gene polymorphism was determined by polymerase chain reaction with restriction of reaction products in 328 CLL patients and 271 age- and sex-matched controls. Immunoglobulin heavy chain variable (*IGHV*) gene mutation status was determined in 321 CLL patients. **Results.** In the control group the study of polymorphisms' distribution showed an association with signs of dyslipidemia and the proportion of GG carriers among persons with elevated triglycerides (TG>2. 1 mmol/l), low-density lipoproteins (LDL>2. 2 mmol/l), or total cholesterol (TC>6. 5 mmol/l) level was higher than among persons with normal levels of these parameters (3. 8% vs 19. 1%, p=0. 001; 4. 2% vs 13. 8%, p=0. 013; and 3. 8% vs 17. 8%, p=0. 025, correspondingly). The distribution of rs6449182 *CD38* genotypes in CLL patients was as follow: CC - 168 cases (51. 2%), CG - 117 cases (35. 7%), GG - 43 cases (13. 1%), and G allele frequency of 0. 31. In comparison with controls who did not have abnormalities in TC, LDL, and TG level an associations between GG genotype and CLL risk in a whole group of CLL patients (OR=3. 92, 95% CI 1. 37-11. 19; p=0. 006), in patients with mutated *IGHV* genes (OR-3. 35, 95% CI 1. 04-10. 76, p=0. 03), and in patients with unmutated *IGHV* genes (OR=4. 03, 95% CI 1. 37-11. 78, p=0. 006) were significant. In contrast, the genotype distributions in CLL patients and controls with abnormalities in TC, LDL, and TG level were similar (p>0. 05). **Conclusions.** we hypothesize that rs6449182 of *CD38* may contribute to the increased CLL risk through generation in GG carriers of increased number and increased diversity of lipid neoantigens due to more frequent dyslipidemia. These neoantigens may be additional antigenic stimules for B-cells prior or after neoplastic transformation. ReferencesJamroziak K, Szemraj Z, Grzybowska-Izydorczyk O, Szemraj J, Bieniasz M, Cebula B, Giannopoulos K, Balcerczak E, Jesionek-Kupnicka D, Kowal M, Kostyra A, Calbecka M, Wawtzyniak E, Mirowski M, Koedek R, Robak T. CD38 gene polymorphisms contribute to genetic susceptibility to B-cell chronic lymphocytic leukemia: evidence from two case-control studies in Polish Caucasians. Cancer Epidemiol Biomarkers Prev 2009; 18: 945-953. Aydin S, Rossi D, Bergui L, D'Arena G, Ferrero E, Bonello L, Omede P, Novero D, Morabito F, Carbone A, Gaidano G, Malavasi F, Deaglio S. CD38 gene polymorphism and chronic lymphocytic leukemia: a role in transformation to Richter syndrome? Blood 2008; 111: 5646-5653.

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AUTOCRINE IL-6 PRODUCTION CORRELATED WITH SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS

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Signal transducer and activator of transcription 3 (STAT3) proteins have been found to play an important role in cancer cell survival and proliferation. The activation of STAT3 signalling pathways require interleukin-6 (IL-6) and tyrosine kinase (JAK2) phosphorylation. Constitutive activation of the IL6/JAK2/STAT3 pathway is contributed to chemo-resistance in Chronic Lymphocytic Leukaemia (CLL). Previous studies have shown that in patients with CLL elevated levels of serum IL6 correlate with adverse disease features and shorter survival. We investigated the relationship of CLL cell autocrine IL-6 production, STAT3 activation and apoptosis resistance. 36 CLL patients with high count of lymphocyte have been investigated and western blot, flowcytometry, gene transfection, fluorescent microscopy techniques have been used for this study. The median autocrine IL6 production in cultured CLL cells was 9. 23pg/ml (range 0. 2-38pg/ml); the patients with higher autocrime IL-6 production (≥9. 23 pg/ml) exhibited a significant good survival in vitro compare with lower autocrine IL-6 production (< 9. 23pg/ml) patients (p < 0. 00001). as compared with stage A patients (6. 1pg/ml, range 0-23pg/ml) (p=0. 02). Patients with high autocrine IL6 production exhibited a high ratio of phosphorylated STAT3/ total STAT3, indicating a high level of STAT3 activation and apoptosis resistance. Activation of the IL6/JAK2/STAT3 pathway by exogenous IL6 led to increased expression of anti-apoptotic proteins Mcl-1 and Bcl-xl. STAT3 activation also protected mitochondrial function during apoptosis: inhibiting cytochrome C release (p=0. 004), mitochondrial membrane potential collapse (p=0. 0004) and ROS generation (p=0. 0007). Finally, STAT3 activation led to increased resistance of CLL cells to spontaneous apoptosis (15% range 2-36%) (p=0. 00008). This study demonstrates that higher CLL autocrine IL6 production correlates with STAT3 activation and apoptosis resistance in CLL. The IL6/JAK2/STAT3 signal pathway may reveal new therapeutic targets.

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CLOPIDOGREL (PLAVIX) INDUCES IN VIVO REDUCTION OF NORMAL B CELLS AND IN VITRO APOPTOSIS OF B CLL CELLS. POSSIBLE ROLE OF AKT EXPRESSION.

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Background. Clopidogrel, widely used in cardiology, is a drug which inhibits the ADP receptor on platelets. Very few patients using this drug suffer also from CLL.

We had the opportunity to follow 2 patients with this association and were surprised to observe a 50% reduction of their peripheral lymphocyte count. **Aims.** To search a possible effect of clopidogrel on B lymphocytes in vitro. **Methods.** We performed the following studies: 1. Determination of B cell count defined by expression of CD19, CD20 on flow cytometry, before and after 4 to 6 weeks use of *plavix*. Twenty patients were selected at the Cardiology Unit and blood was collected after informed consent. 2. Evaluation of apoptosis of B CLL cells induced by clopidogrel in vitro in twenty non-treated CLL patients. Blood was collected after informed consent; lymphocytes were separated on ficol then studied for apoptosis using the Annexin V assay by flow cytometry before and after addition of clopidogrel. The dosage of 20 mcg per 4 x10^6 cells represents approx. the concentration of treated patients by the drug. Higher dosages of 50 and 100 mcg were used for comparison. **Results.** 1. B lymphocyte counts were significantly reduced in non CLL patients treated by *plavix* for cardiac reasons. Mean CD19 was 15. 5% before *vs* 10% after (p=0. 015) and mean CD20 was 15% before *vs* 9% after 4 to 6 weeks of *plavix* treatment (p=0. 014). 2. Addition of *plavix* in CLL cells vitro induced an increased apoptosis: mean from 10 to 20% (p=0. 004) with 20 mcg and the higher dosage of 50 and 100 mcg induced a high level of necrosis. Since the mechanism of platelet activation is inhibited through the Akt promoter, we studied the expression of Akt on B CLL cells using anti-AktMoAb(ENCO) after cells permeabilization. We found a significant reduction of the expression of Akt from 63% to 22% after addition of 20 mcg clopidogrel. **Conclusions.** We demonstrated that non-CLL patients treated with clopidogrel (*plavix*) had a significant reduction of their B cells after 4-6 weeks of treatment. The addition of pharmaceutical dosage of clopidogrel induced a significant increase of in vitro apoptosis in CLL cells, possibly explaining the lymphocyte reduction count showed in our two B CLL patients who received *plavix* for cardiac reasons. Since we showed a reduction of the Akt expression in CLL cells after addition of clopidogrel in vitro, we suggest that inhibition of Akt is possibly involved in our findings.

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TCL1 AND PAKT EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: CORRELATION WITH CLINICAL, LABORATORY FEATURES AND OUTCOME

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Background. Deregulation of TCL1 oncogene has implicated in the pathogenesis of the aggressive form of B CLL with however unknown mechanism of action. Akt pathway has been proposed to be a possible target of TCL1 oncogene. AIMTo evaluate TCL1 expression in a series of CLL patients (pts), correlate its expression with the clinical and laboratory data as well as other prognostic factors, and investigate the possible involvement of Akt pathway in TCL1 mechanism of action. **Patients and Methods.** 69 pts diagnosed with CLL/SLL by using standard criteria included in this study. Baseline clinical and laboratory features were recordedTCL1 as well as ZAP70 and pAkt expression were evaluated by immunohistochemistry (IHC) with standard techniques in bone marrow paraffin embedded sections(61pts) and lymph node sections(8pts). TCL1 positive cases were confirmed by flow cytometry too. **Results.** 69 pts (42male) with median age of 61 years (35-85) included in the study. 53 pts(77%) had disease stage A, 11 (16%) B, and 5(7%) C, according to Binet staging system. IgVH mutational analysis was performed in 38 pts and 21/38 were unmutated. FISH analysis for 11q del and 17p del were evaluated in 33 και 36 pts respectively. In 3 pts 11q del was detected while one patient was positive for 17p del. CD38 detection was performed in 43 pts (19% were CD38 positive). Forty-five (65%) pts were TCL1 positive and 37 pts (54%) were ZAP70 (+). The majority of TCL1 positive CLL patients showed strong positivity whereas TCL1 localization was mainly in the cytoplasm. In addition, pAkt expression was observed only in TCL1 positive CLL pts and it was localized both in membrane and cytoplasm. Thirty-one out of 42(73%) male pts presented TCL1 positivity while 14 out of 27 females were TCL1(+) (p:0. 03). 32/37 ZAP70(+) pts were also TCL1(+) against 13/32 ZAP(-) (p:<0,001). Moreover 18 out of 21(86%) unmutated pts expressed TCL1 while 8 out of 17(47%) mutated pts were TCL1(+) (p:0. 03). TCL1 expression was more frequent in pts with non nodular pattern of bone marrow infiltration. Proliferation centers(PCs) were recognized in 20 bone marrow sections and in all lymph node sections. TCL1 was strongly expressed in the small cell compartment of the PCs but it was negative/weak in the mitotically active paraimmunoblasts in these areas. No differences in 7-year treatment free interval and 10 year overall survival were observed between TCL1(+) and TCL1(-) pts. **Conclusions.** TCL1 expression was strongly correlated with markers of the pre-germinal center subset of CLL (unmutated VH genes status and higher ZAP 70 expression levels), findings that were in accordance with previous published data. TCL1 expression was not correlated with any of the clinical predictive factors. TCL1 was correlated with male gender. No correlation was found between TCL1 expression and patients' survival. pAkt expression was observed exclusively inTCL1positive cases. pAkt coexpression with TCL1 may suggest a common mechanism of action.

1270

ASSOCIATION BETWEEN MBL2 GENE POLYMORPHISMS AND PREDISPOSITION TO THE DEVELOPMENT OF INFECTIOUS DISEASE ON CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background. Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the western hemisphere. It is characterized by a progressive accumulation of small mature B lymphocytes on the peripheral blood and lymphoid tissues. Infectious disease represents the main cause of death on CLL patients. *MBL2* gene codes the mannose-binding lectin (MBL), an important constituent of human immune defense. The MBL protein act as initial factor of the lectin pathway of the complement system and its deficiency appears to predispose to serious infection. It was been reported that polymorphisms on *MBL2* gene could be associated to a higher risk to the development of infectious disease. **Aims.** We aimed to define how *MBL2* gene polymorphisms varied among CLL patients and clarify the relationship between these genetic alterations with infections and others clinical and laboratorial features. **Methods.** Patients diagnosed with CLL by immunophenotyping had their records analyzed for clinical and laboratorial data collection. Real-time PCR techniques were used to identify the polymorphisms G-550C and G-221C at promoter region and the presence of *O* allele, which result from polymorphisms at exon 1 region in *MBL2* gene. We also determined the *MBL2* secretor haplotypes. **Results.** A total of 116 patients with CLL were enrolled in our case-control study. Forty-four patients presented laboratorial and clinical signs or symptoms of infectious diseases after diagnosis. The control group consisted of 72 patients with absence of infectious events. Herpesvirus and respiratory tract infections represented the most common forms of infections on our cohort. The follow-up time was significantly lower (p=0. 034) in the control group. Binet stage (p=0. 001) and splenomegaly (p=0. 002) presented high association on the case group compared with the control group. However, the variant alleles and genotypes of *MBL2* gene promoter and exon 1 regions do not demonstrate influence as genetic modulator of infection risk on our cohorte. MBL levels correlate with *MBL2* haplotypes also showed no significant association with infection. **Summary and Conclusions.** From all parameters analyzed in our study, only follow-up time, Binet stage and splenomegaly showed significant influence as modulators for infectious diseases prevalence on patients with CLL. The genetic heterogeneity among different populations may explain the failure in reproducibility of previous gene association studies, and warrants further studies in worldwide collaboration to determine the actual relevance of findings involving genetic polymorphisms in *MBL2* gene and their influence on the susceptibility to infections in CLL.

1271

ENDOGLIN MRNA EXPRESSION IS INCREASED IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA WITH POOR PROGNOSTIC MARKERS

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Background. Angiogenesis is considered to play an important role in pathogenesis and progression of chronic lymphocytic leukemia (CLL). Endoglin (CD105), a member of transforming growth beta receptor family, is one of key angiogenic molecules and its elevated expression has been reported in hematological malignancies as well as solid tumors. However, data regarding endoglin expression in CLL cells are very limited. **Aims.** To evaluate prognos-

tic significance of endoglin mRNA expression in malignant lymphocytes from CLL patients and its relationship to traditional and modern prognostic factors. **Methods.** Endoglin mRNA levels were analyzed in CD19+ cells of 49 untreated CLL patients by real-time quantitative PCR and normalized for differences in RNA concentration in each sample by quantitation of housekeeping gene hypoxanthine phosphoribosyltransferase 1 (HPRT1). ZAP-70 and CD38 expression were analyzed by flow cytometry; IgVH mutation status was assessed according to usual methodology using cDNA and IgBLAST database. **Results.** We found significant association between high endoglin mRNA levels and unmutated IgVH genes (n= 38, p < 0. 001), high CD38 expression (n = 46, p = 0. 005) and high ZAP-70 expression (n = 45, p = 0. 043). In addition, endoglin expression was significantly higher in patients with progressive disease (n = 49, p = 0. 011) and significantly lower in Rai stage 0 (n = 49, p = 0. 004). **Conclusions.** Our pilot data show that endoglin mRNA is differentially expressed in malignant lymphocytes of CLL patients and high endoglin expression is associated with unmutated IgVH and high expression of CD38 and ZAP-70; thus, it seems to be associated with poor prognosis of CLL. Moreover, expression of endoglin might play an important role in the biology of CLL progression, because it is elevated in patients with Rai stage I - IV and those with progressive disease. Supported by research project MZO 00179906 from Ministry of Health,Czech Republic.

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WITHDRAWN

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ANTIPROLIFERATIVE AND APOPTOTIC EFFECTS OF RESVERATROL ON CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background. Chronic lymphocytic leukemia (CLL), defined by accumulation of pathogenic B cells. CLL has a complex biology due to interplay of inherited, enviromental and host factors. Therefore, its complicated etiology makes treatment of CLL difficult. Recently, finding new treatment agents or development of novel treatment strategies have been paid attention. Resveratrol is a natural polyphenolic phytoalexin and found in a wide variety of plants like skin of red grapes, peanuts, and mulberries. It was previously shown that resveratrol has antioxidant, antiviral, chemopreventive and antitumoral activities. The anticarcinogenic effects of resveratrol were investigated on a variety of cancer types like colon, lung, breast, pancreas and chronic and acute myeloid leukemia. **Aims.** In this study, we aimed to investigate cytotoxic, cytostatic and apoptotic effects of resveratrol on 232B4 chronic lymphocytic leukemia cells. **Methods.** Antiproliferative effects of increasing concentrations of resveratrol on 232B4 cells were determined by MTT cell proliferation assay. Apoptotic effects of resveratrol were determined by changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential, and Annexin-V staining by flow cytometry. Effects of resveratrol on cell-cycle progression of 232B4 cells were examined using DNase-free RNase and propidium iodine by flow cytometry. **Results.** There were significant decreases in proliferation of 232B4 CLL cells exposed to increasing concentrations of resveratrol (1-500 μM) for 72 hours and the IC50 value of resveratrol was found as 27 μM. Resveratrol increased caspase-3 enzyme activity, induced loss of MMP and triggered apoptosis in 232B4 cells in a dose-dependent manner. There were 2, 16, 38, 49 and 107% increases in caspase-3 enzyme activity; 3, 5, 99, 1792 and 3105% increases in loss of MMP; and 2, 8, 133, 350 and 382% increases in apoptotic cells in response to 0. 5, 1, 10, 50 and 100 μM resveratrol, respectively, compared to untreated 232B4 cells. Treatment of 232B4 cells with resveratrol (0. 5-100 uM) resulted in a significant increase in G0/G1 phase while decreased S phase cell population as compared to untreated controls. **Summary and Conclusions.** Taken together these results showed that resveratrol inhibits proliferation and cell cycle progression of 232B4 cells in addition to induction of apoptosis in a dose-dependent manner. Resveratrol triggers apoptosis by inducing caspase-3 enzyme activity and forming pores on the mitochondrial membrane.

1274

EVALUATION OF SERUM FREE LIGHT CHAINS (FLC) AS A NEW PROGNOSTIC FACTOR FOR CHRONIC LYMPHATIC LEUKEMIA B (CLL-B) PATIENTS

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Background. Serum FLC quantitation has prognostic value in multiple myeloma, MGUS (monoclonal gammapathy of unknown significance) solitary plasmocytoma and primary amyloidosis; FLC has been included in response criteria for myeloma and amyloidosis. Several recent studies have described FLC abnormalities in 39-54% of CLL-B patients and their association with poor survival and fast progression. **Aims.** We intend to compare the clinical outcome of our CLL-B patients with or without abnormal serum FLC. **Patients and Methods.** We analyzed restrospectively 58 CLL-B patients diagnosed between 1995 and september 2011. They were 23 female and 35 male patients of a median age of 70 years (44-91). FLC were determined in serum samples stored at diagnosis; a nephelometry immunoassay was used. Monoclonal FLC elevation was defined as elevated FLC (kappa >19. 4mg/l or lambda>26. 3 mg/l) with an abnormal FLC ratio(0. 25-1. 65); polyclonal FLC elevation was defined as elevated FLC with a normal FLC ratio;patients with normal FLC but an abnormal FLC ratio were evaluated as abnormal FLC ratio. 81% of patients were classified as Binet stage A, 15. 6% as stage B and 3. 4% as stage C. Cytogenetic analysis was evaluable in 67. 2% of cases:10. 2% of them were hig risk (del17positive), 15. 3% had trisomy 12, 33. 3% were del13q positive and 41,3 % were normalCD38 was determined in 91. 3% of cases and was negative in 86. 8%ZAP70 had been performed in just 29. 3% of patients and was positive in 41. 2% of them. 77. 6% of patients had a normal LDH and 65% a normal B2 microglobulin. These clinical and laboratory measures were evenly distributed in both groups of patients, with normal or anomalous FLC, except for CD38 (more frequently positive in the abnormal FLC group). **Results.** With a median follow-up of 59. 5 months (6-216), 37. 9% of patients required treatment and overall mortality was 8. 6%. Abnormal FLC were found in 48. 2% of patients (18 monoclonal, 7 polyclonal and 3 abnormal FLC ratio)None of the patients diagnosed of Richter syndrome had abdnormal FLC. With a median follow-up of 65. 5 months (9-189), mortality was 10. 7% for the group of patients with abnormal FLC and 6. 7% for the normal FLC group with 57m of follow up; we did not find a statistical significant difference. The percentage of patients requiring treatment was 35. 7% for the abnormal FLC group and 40% for the normal FLC group; it was not significantly different . When analyzed separately, patients with monoclonal FLC elevation were not significantly associated with poor survival nor with early treatment requirement, compared with normal FLC patients. **Summary and Conclusions.** In our cohort of CLL-B patients, FLC elevation was neither associated with an inferior survival nor with a shorter time to treatment as had been previously published . So we cannot assess for certain the usefullness of this assay as a biomarker or prognostic factor in CLL-B. More studies with a higher number of patients and longer follow up may be needed

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CD38 GENE POLYMORPHISM IS NOT ASSOCIATED WITH CHRONIC LYMPHOCYTIC LEUKEMIA SUSCEPTIBILITY IN PERSONS EXPOSED TO IONIZING RADIATION DUE TO THE CHERNOBYL NPP ACCIDENT

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Background. Chronic lymphocytic leukemia (CLL) is the most common leukemia among adults in Ukraine, as well as in Western Europe and USA. CLL also was shown to be the most common form of leukemia in the clean-up workers of the Chernobyl Nuclear Power Plant (NPP) accident, although impact of ionizing radiation (IR) in CLL development is not proven. Recent studies suggest the association of some genotypes with elevated risk of CLL. Particularly, CD38 rs6449182 (184 C>G) single nucleotide polymorphism (SNP) was reported may contribute to CLL predisposition, affecting CD38 expression. Association of variant CD38 rs6449182 G allele with more aggressive CLL phenotype also was shown. CD38 antigen is well-known negative prognostic marker in CLL, which is supposed to be also one of the key elements in the disease pathogenesis, ruling proliferation/survival signals in leukemic cells. **Aims.** The aim of the study was to evaluate whether CD38 rs6449182 polymorphism influence predisposition to CLL in persons exposed to ionizing radiation. **Methods.** The CD38 rs6449182 genotypes were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using primers

proposed by Aydin S. et al. (Blood, 2008). The 128-bp PCR product was digested with *Pvu*II (Fermentas), resolved on 4% agarose gel, and analyzed. **Results.** Genotyping of CD38 rs6449182 SNP was performed in 77 IR-exposed CLL patients and in the group of 136 IR-exposed controls. CLL patients and IR-exposed controls were comparable by gender and age (67 M/10 F, mean age 57. 5+8. 9, range 39-87 years vs. 120 M/16 F, mean age 61. 5+ 8. 3, range 37-86 years). CD38 rs6449182 genotypes distribution was in accordance with Hardy-Weinberg equilibrium in both groups. The frequency of variant CD38 rs6449182 G allele in CLL group was comparable to health controls (0. 33 vs. 0. 31, P=0. 79). The distribution of genotypes among CLL patients was found as CC - 42. 9%, CG - 48. 1%, GG - 9. 1%). It also did not differ significantly from that found in control group (CC - 44. 9%, CG - 48. 5%, GG - 6. 6%, P=0. 63). To evaluate possible clinical/biological associations of CD38 rs6449182 SNP, we analyzed range of prognostically important variables in CLL group depending on CD38 genotype. No correlation was found between the CD38 rs6449182 genotypes and age, sex, Binet stage at diagnosis, initial WBC count. Furthermore, there were more cases with mutated IGHV (immunoglobulin heavy-chain variable region) genes among variant rs6449182 G allele carriers in comparison with rs6449182 CC homozygotes (44. 2% vs. 21. 2%, P=0. 036). **Conclusions.** Our results suggest that CD38 rs6449182 SNP does not influence the risk of CLL in persons exposed to IR. Variant CD38 rs6449182 G allele is not associated with poor risk prognostic markers in IR-exposed CLL patients.

1276

SERUM LEVEL OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF), BUT NOT VEGF RECEPTOR EXPRESSION, IS ASSOCIATED WITH THE PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. VEGF, a potent pro-angiogenic factor, regulates vascular proliferation through its receptor (VEGFR) activation. Chronic lymphocytic leukemia (CLL) cells express VEGFR and synthesize and secrete VEGF (sVEGF) and might be involved in the pathophysiology of CLL. sVEGF levels have been associated with advanced clinical stage and disease progression but the prognostic value of VEGFR is still controversial. **Aims.** To determine in CLL patients, serum concentration of sVEGF and the expression of VEGF in leukemic cells, to correlate the results with the clinical and biological prognostic factors, and to evaluate their effect on disease progression. **Methods.** Seventy seven previously untreated CLL patients (61% male; median age 70 years, range 38-88 were studied). Most patients were Binet A (n=45, 58%). In addition, 14 healthy volunteers (6 males; median age = 54 years, range: 38-79) were evaluated as controls. VEGFR expression was evaluated in peripheral B lymphocytes by flow cytometry using the Fluorokine ® Biotinylated Human VEGF (R&D systems Inc., Minneapolis, MN, USA) kit. Results were expressed as mean fluorescence intensity (MFI). sVEGF was also determined, in 36 patients, by ELISA, using the Quantikine ® Human VEGF Immunoassay kit (R&D systems Inc., Minneapolis, MN, USA). Results were compared according to Binet clinical stage (A vs. B or C), absolute lymphocyte count (< vs. ≥30x10^9/L), LDH (< vs. ≥276UI/L), β2 microglobin (< vs. ≥2,7mg/L), expression of CD38 (< vs. ≥30%), *IgVH* mutational status (mutated vs. unmutated). Progression-free survival was determined by the Kaplan Meyer method. Median follow-up time was 40 months (range: 2 - 41). **Results.** Median MIF of VEGFR was lower in CLL patients (147, range: 8-1489) as compared to controls (373, range: 222 - 1072) (p<0,0001). Median sVEGF was similar between patients and controls. No significant differences regarding Binet stage, lymphocyte count, LDH, CD38 expression and *IgVH* mutational status were observed between patients with higher or lower VEGFR levels (IMF < or ≥141). There was also no difference in progression-free survival at 3 years between the groups. Patients with high sVEGF values (≥ 400pg/mL) had more frequently high LDH levels (63% vs. 37%, p=0,04), high lymphocyte count (65% vs. 35%, p=0,04) and a trend to present more advanced disease (66% vs. 33%, p=0,08), as compared to those with low sVEGF values. No correlation between sVEGF levels and*IgVH* mutational status, CD38 expression and β2 microglobin was observed. Patients with higher sVEGF levels had a worse progression-free survival at 3 years (68%) as compared to those with lower sVEGF levels (92%, p=0. 03). **Conclusions.** High levels of sVEGF are associated with unfavorable prognosis and worse progression free survival in CLL patients. Correlation between VEGFR expression and sVEGF deserves further investigations and may contribute to understand the pathophysiology of the disease.

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THE RECEPTOR TYROSINE KINASES ROR1 AND ROR2 EXPRESSION PATTERNS IN LYMPHOID AND MYELOID MALIGNANCIES

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Background. ROR1, a receptor tyrosine kinase, is expressed in CLL and other subtypes of lymphoma. **Aims.** The aim of this study was to further characterize expression of ROR1 and the other member of the ROR family, ROR2, in lymphoid and myeloid malignancies using self-produced antibodies and commercial anti-ROR1 as well as anti -ROR2 antibodies. **Results.** Normal PBMC and reactive lymph nodes were negative for ROR1 and ROR2. A significantly higher and uniform surface expression of ROR1 was found in CLL compared to the other tumors (p=0. 02-<0. 001). Lymphoma subtypes (MCL, MZL, DLBCL, FL), myelomas, ALL and myeloid leukemias showed a variable staining and considerable inter-patient variability. The lowest proportion of ROR1+ cells was found in follicular lymphomas whereas CLL and CML had significantly higher numbers of ROR1+ cells. Longitudinal follow-up of individual CLL patients revealed that the fraction of ROR1+ cells remained stable over time in non-progressive patients but increased when the disease progressed (p<0. 05). ROR2 was not detected in hematological malignancies. Self-produced anti-ROR1 mAbs detected more ROR1+ cells than the commercial anti-ROR1 antibody (p<0. 05-<0. 01). **Conclusions.** In summary, ROR1 but not ROR2 is expressed in different hematological malignancies with a variable staining pattern ranging from very high (CLL) and high (CML), to intermediate (myeloma and DLBCL) or low (FL).

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QUERCETIN INDUCED APOPTOSIS IN 232B4 CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background. Chronic lymphocytic leukaemia (CLL) is the most frequent form of leukemia in adult population (22-30% of all leukaemia cases) in the Western world. Cell death evasion and progressive accumulation of B cells in the blood, bone marrow, lymph nodes, and spleen are the major events in CLL pathogenesis, At present, there is no curative therapy for CLL due to variable prognostic factors, course of disease and even advanced age of many patients. Quercetin is an important dietary flavonoid, present in different vegetables, fruits, seeds, nuts, tea and red wine. Quercetin is used as potential chemopreventers or chemotherapy reagents on a variety of cancer types like colon carcinoma, glioma and non-small cell lung cancer. The mechanisms of quercetin-induced apoptosis vary from one cancer type to other. **Aims.** In this study, we aimed to examine antiproliferative and apoptotic effects of quercetin on 232B4 CLL cells. **Methods.** Antiproliferative effects of step-wise increasing concentrations of quercetin on 232B4 cells were determined by MTT cell proliferation assay. Apoptotic effects of quercetin were determined by changes in caspase-3 enzyme activity and Annexin-V staining by flow cytometry. Cytostatic effects of quercetin on 232B4 cells were examined using DNase-free RNase and propidium iodine by flow cytometry. **Results.** There were significant decreases in proliferation of 232B4 CLL cells treated with increasing concentrations of quercetin (0. 1 to 100 μM) for 72 hours and the IC50 value of quercetin was calculated from cell proliferation plots and found as 24 μM. Increasing concentrations of quercetin increased caspase-3 enzyme activity and triggered apop-

tosis significantly. There were 3, 12, 38 and 47% increases in caspase-3 enzyme activity in response to 0. 1-, 5-, 10- ve 50 μM quercetin, respectively, as compared to untreated 232B4 control. The same concentrations of quercetin induced apoptotic cell death by 56, 133, 160 and 297% as compared to untreated control cells. Interestingly, treatment of 232B4 cells with quercetin (0,1 to 50uM) resulted in a significant increase in G0/G1 phase as compared to untreated controls showing that quercetin not only induces apoptosis but also arrest the cell cycle progression. **Summary and Conclusions.** Taken together, these data reveal that quercetin inhibits proliferation and cell cycle progression and induces apoptosis in a dose-dependent manner in a CLL cells.

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STROMAL MICROENVIRONMENT LYMPH NODES IN CLL

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Background. Role or the lymphoid microenvironment in regulation of development lymphoid precursors is known. However the state of lymphoid stromal cells when lymphocyte proliferation is impaired continues to be studied insufficiently. In the work presented structural changes stromal elements lymphoid nodes and impaired capability of stromal cells lymphoid tissue secrete IL-4, IL-6 and TNF- α in CLL. **Aims.** To study cellular and humoral factors of stromal microenvironment lymph nodes in patients with CLL before treatment. MethodsStromal cells of biopsy lymph nodes seventy-five patients with CLL was studied using morphology, immunohistochemistry method. Proliferative activity of stromal cells was evaluated in organ culture lymphoid tissue. Concentration IL-4, IL-6, TNF- α in culture medium has been studied. **Results.** Increase microvessels and reticular cells in lymph nodes were bound. Reticulin staining showed fibrosis in 53 patients. In vitro increased proliferative activity stromal cells lymphoid tissue was established in all patients. The production of humoral factors by lymphoid microenvironment changed. We found increase IL-4, IL-6, TNF- α in condition medium after two weeks of culture in the presence of stromal cells. The stromal cells of tissue section of lymph nodes contained lymphocytes in cytoplasm. The process emperipolesis in adherent cells of lymph nodes cultures was seen too. **Conclusions.** The changes stromal microenvironment of lymph nodes can be one of the main pathogenetic factors in the impairment of lymphoid cells development in CLL.

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EVALUATION OF 6Q21 AND 8Q24 ABERRATIONS AS MARKERS FOR PROGRESSION AND EVOLUTION OF CLL

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Background. Deletion 6q21 and 8q24 amplification are associated with poor prognosis in lymphoproliferative disorders that should be discovered to identify patients with poor prognosis. **Aims.** to detect 6q21/c-Myc aberrations in addition to the common aberrations in CLL patients and correlate these chromosomal abnormalities to the standard prognostic markers and clinical outcome of the disease.

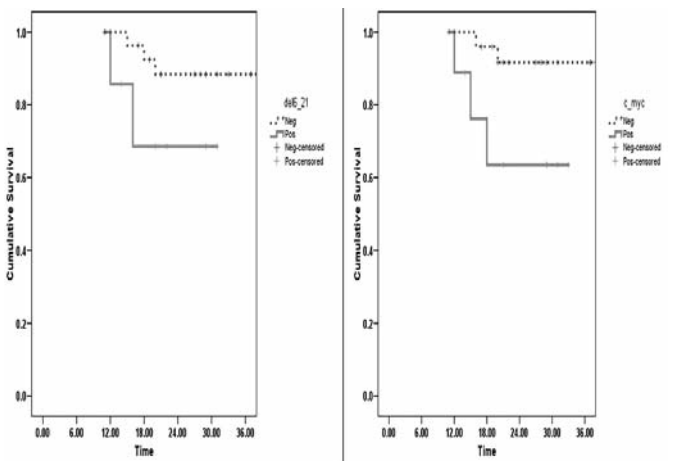

Figure 1. Kaplan Meier curves showing difference in event free survival of CLL patients having 6q21 deletion and c-Myc amplification (8q14) compared to negative ones.

Methods. Fluorescence in situ hybridization (FISH) technique using locus-specific identifier (LSI) 6q21 deletion /c-Myc (8q24), was applied on 75 B-CLL patients, in addition to the routine panel of probes (LSI 13q14,17p13 and centromeric 12) together with Immunophenotyping using the routine panel for lymphoproliferative disorder. **Results.** 6q21 deletion were detected in 14/75 (18. 7%) patients while c-Myc amplification were positive in 20/75 (26. 7%) patients. Each of these aberrations was associated with a shorter disease free survival. 6q21 deletion was significantly associated with low Hb level, high total leucocytic count, low platelets count, short LDT, advanced Rai clinical staging and higher expression of CD38. c-Myc amplification had statistically significant association, with lower Hb level, high TLC and shorter LDT. Deletions in 13q14 were 29/75 (38. 7%), trisomy 12 were in 18/75 (24%), and finally deletion 17 q (P53) were in 6/75 (8%). Higher incidence of 13q14 deletion was detected in patients with good outcome. On the contrary, trisomy 12 was significantly associated with a number of poor laboratory prognostic parameters and poor outcome. Disease outcome showed statistical significant association with some standard prognostic markers as old age, sex, advanced Rai staging, LDT, and CD38 expression. **Conclusions.** Deletion 6q21 and amplification c-Myc identify subgroup of CLL patients, associated with poor prognosis. These aberrations should be investigated before designing management protocols for patients presenting with advanced disease or poor prognosis early stage disease.

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EXPRESSION OF NATURAL KILLER CELL ACTIVATING RECEPTORS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Backgroud. Recent advances in chronic lymphocytic leukemia (CLL) treatment, more particularly *via* upfront use of anti-CD20 monoclonal antibodies, have prolonged patient progression-free survival. Nonetheless, apart from allogeneic stem cell transplantation, no curative treatment is available. One possible explanation for the lack of cure in CLL could be defective immune antitumor response. Due to abnormal human leukocyte antigen (HLA) class I molecule expression, CLL cells escape from specific T-lymphocyte immunity but thus should be the target for innate NK-mediated immune response. **Aims.** Defective NK cytotoxicity due to decreased expression of the Natural Cytotoxicity Receptors (NCR) NKp30/NCR3, NKp44/NCR2 and NKp46/NCR1 has been described in hematological malignancies such as acute myeloid leukemia (AML). This prompted us to focus our attention on NCR expression on NK from CLL patients. **Methods.** NK cell analysis as performed by flow cytometry using NK cells from CLL patient (obtained after informed consent) in comparison with age-matched donors. NK cells were defined as CD3-CD56+. **Results.** Although we failed to detect any difference between CLL patients and healthy age-matched controls, a precise analysis of clinical data showed a correlation between decreased NCR expression and poor prognosis factors such as low hemoglobin (Hb) level, high (>30 G/L) lymphocyte number or elevated C reactive protein (CRP). **Conclusions.** Together, these observations support the rationale for restoration of normal NK functions in CLL patients, putatively *via* the use of immune therapy protocols that already have demonstrated some benefit in AML such as IL-2 plus histamine dihydrochloride.

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T PERIPHERAL CD26 NEGATICE CELLS IN CD4 POSITIVE LONG TERM LYMPHOCYTOSIS

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Introductions. Flow cytometric (FC) immunophenotyping is a powerful modality in the characterization of lymphocytosis but it can be insufficient, in detecting the nature of T cell expansions. Seen that T neoplasms can often show aberrant phenotype, from 2009 we have analyzed in patients with persistent asintomatic T lymphocytosis, the expression of different antigens. **Methods.** 30 asymptomatic subjects (average age 62 years) with persistent peripheral lymphocytosis with T proliferations (>4560 mm^3 T cells) were followed up for three years; antigens such as CD3, CD4, CD7, CD8, and TCRV were tested. Over the pan-T-cell antigens, we have investigated the CD26 which represents a molecule expression with multiple biological functions and demonstrated utility in the diagnosis of T cell disorders (it is preferentially restricted to the CD4+ helper/memory population and many reports show its absence is characteristic of circulating Sézary cells). Cells from peripheral blood were stained with monoclonal antibodies and analyzed by FC to assess the expression of T cell antigen. In samples with phenotypic alterations we have studied the TCRV and

performed molecular analysis for TCR gamma gene clonal rearrangement. **Results.** over the antigen panel positive for the phenotype $CD3^+$ $CD4^+$ $CD8^-$ and $CD45R0^+$,the most commonly observed aberrancy was the lack or the very low expression of CD26 subset (100% of cases) while CD7 subset was variable as it was absent/low in 50% of samples. All, moreover, showed alterations variously in the TCRV repertoire and their clonal nature was confirmed by the demonstration of TCR rearrangement. After this 3 yrs follow up 2 out of 14 (14%) of the subjects have been diagnosed as Sezary syndrome. **Conclusions.** Asymptomatic persistent clonal lymphocytosis with immunophenotype $CD4^+$ $CD26^-$ defined as monoclonal T lymphocytosis (MTL) should be monitored carefully as the absence of CD26 expression may be suitable of the progression towards T malignant lymphoprolipherative syndromes such as SS

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C-KIT MUTATION DETECTION IN TUNISIAN PATIENTS WITH NEWLY DIAGNOSED MYELOGENOUS LEUKEMIA: PREVALENCE AND PROGNOSTIC SIGNIFICANCE

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Background. *C-KIT* gene encodes a class III tyrosine kinase receptor. Specific somatic mutations in this gene were associated with many diseases. **Aims.** In this study we investigated the prevalence of *C-KIT* mutations in patients with chronic and acute myelogenous leukemia (CML and AML) and their prognostic significance. **Methods.** A total of 157 subjects was included in the present study (84 patients with CML, 33 with AML and 40 healthy controls). All patients were analyzed at the first diagnosis. The *C-KIT* mutations were screened by PCR and direct sequencing. **Results.** Our results have reported the presence of G/A transition at codon 796 which is associated with R796K protein variation. This mutation is described for the first time and detected at 21. 42 % in CML subgroup and it was absent for AML and healthy controls. However, we have not found any correlation between this mutation and clinical parameters such us molecular response to Gleevec. **Conclusions.** In conclusion we retain that *C-KIT* gene is highly mutated in CML but its role as a prognostic factor needs to be more elucidated.

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DISTRIBUTION OF CYTOGENETIC ABNORMALITIES IN CHRONIC LYMPHOCYTIC LEUKEMIA IN BLIDA

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Background. To clarify the cytogenetic characteristics of chronic lymphoid leukemia (CLL) in our region, a prospective study by fluorescent in situ hybridization (FISH) was conducted. **Aims.** to identify patients with poor prognosis cytogenetic abnormalities. **Materials and Methods.** Over a period of 5 years, 95 cases of LLC have been studied by FISH; collection of peripheral blood on heparin lithium; a culture of 72 hours with PMA / PHA is carried out. The probes used: CEP12,13 q 14 / 13qter, P53 (17p13) / ATM (q 11, 22), 6 q 21 / SE 6. **Results.** The study involved 95 new cases of LLC; 69 men and 26 women with a median age of 61 years (37-80). Matutes score =3 in6 cases (6. 3%), the rest (93. 7%) at 4-5. Binet classification: stage A = 17, stage B = 33, stage C = 45. Recurrent anomalies found in 78 cases (82. 1%):del(13 q 14) in 48 cases (50. 5%), 25 times isolated. Trisomy12 in25 cases (26. 3%), isolated in 13 cases;del(P53) in 17 cases (17. 8%), isolated in 2 cases;del(ATM) in 16 cases (16. 8%), isolated in 3 cases;del(6 q 21) in 11 cases (11. 5%). No abnormalities in 17 cases (17. 9%). According to Binet stage: Stage A (17 cases): 12 present adel(13 q 14), isolated in 10 cases (58. 8%). Trisomy 12: 3 cases. del(P53) in 2 cases. No abnormalities: 2 cases. Stage B (33 cases): no abnormalities in 10 cases (30%); a del (13 q 14) in 14 cases, isolated in 5 cases (15%); a Trisomy12 in8 cases (24. 2%); an del (ATM) in 7 cases (21. 2%), a del (P53) in 2 cases (6%), a del (6q 21) in 5 cases (15%). In the stage C (45 cases): del (13 q 14) in 22 cases, 10 times isolated (22. 2%), in 7 cases associated with del (P53)and with 3 del (ATM) cases and in 2 cases with del (6 q 21); a del (P53) in 11 cases (24. 4%), isolated in one case; a Trisomy12 in 14 cases, isolated in 7 cases (15. 5%); an del (ATM) in 9 cases (20%) isolated in a case; No abnormalities in 5 cases (11%). **Conclusions.** Cytogenetic analysis by FISH is a useful prognostic tool. The frequency of recurrent abnormalities found by FISH in our work is similar to other published series. (82. 1% vs 80%); The del (13 q 14) is a little less common than which was previously reported by others (50. 5% vs. 55 to 63%, respectively); frequency of Trisomy 12 (26, 3 vs 13 to 25%), and the del (ATM) (16. 8% vs 11 to 18%) join those of literature; the frequencies of the del (P53) (17. 8% vs. 7%) and the del (q 6, 21) (11. 5 vs 7%) are higher than those observed in the literature. Thedel(ATM) is found only in the stages B and C; of the 17 cases ofdel(P53), 11 cas (64. 7%) are found at stage C. These data indicate the high frequency of del(P53) in our study, deemed very poor prognosis because of the resistance to therapeutic regimens fludarabine-based.

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CORRELATION BETWEEN ZAP-70 LEVEL AND OTHERS PROGNOSTICS MARKERS IN B- CLL PATIENTS

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Background. ZAP-70 (zeta-associated protein-70kDa) is normally expressed in T cells and NK cells. Recent studies have shown its value as a surrogate marker for the IgVH mutation status in B-CLL. ZAP-70 is very useful for B-CLL prognosis. Using flow cytometry we investigated if there is any correlation between ZAP-70 expression and other prognostic markers in B-Cell chronic lymphocytic leukemia (B-CLL). B-CLL is the most frequent oncoheamatological diagnosis inLatvia within the past 10 years. **Aims.** The levels of ZAP-70, Thymidine kinase (TK), B-2 microglobulin (B2mg), Lactate dehydrogenase (LDH) and CD38 was determined in patients with B-CLL both pre treatment, 6 months and 12 months post initiation of chemo and glucocorticoid therapy. Patients were grouped using the Rai staging system and levels of markers assessed within each stage. B-CLL is the most common oncohaematological disease inLatvia and to be able to rapidly identify and utilize all markers as a prognostic factor in this disease will improve the appropriate selection of more effective treatment. **Methods.** The research group consisted of 120 newly diagnosed B-CLL patients during the period 2008 to 2010. The selection of patients was carried out at the Centre of Haematology inLatvia. ZAP-70 level was determined using the PN 772587 kit from Beckman Coulter, Inc. We had defined three levels of ZAP-70 expression: ZAP-70 interval 0-10 % positivity, reflects low degree of disease activity, 10-20 % positivity reflects medium degree of disease activity, greater than 20 % positivity corresponds to a high degree of disease activity. The level of CD38 was determined using CD38-FITC PN IM0775 from Beckman Coulter, Inc. The cut-off level was defined as 30 % positive. Serum TK levels was determined using an ELISA technique. Serum TK level in healthy donors is less then 50 ng/L. In this method a minimal detection level (sensitivity) of 50ng/L was attained. The parameters LDH, B2mg were determined by using of lumeniscent immunochemocal method Latvian Central Ethics Commission approved the research design and all patients enrolled in this investigation had given informed consent. **Results.** 120 patients were assessed in this study, consisting of 60 females and 60 males; the mean age of patients was 68. The patients were divided into three groups according to ZAP-70 expression: I- ZAP-70 0-10% +ve; II - ZAP-70 10-20% +ve and III - ZAP-70 greater then 20% +ve. **Conclusions.** The correlation between ZAP-70 and CD38 are in all period of control, in groups with 10-20% expression of ZAP-70. There are correlation between TK and LDH in different groups ZAP-70 expression in the period of after 12 month . The pair of prognostic markers ZAP-70/CD38 can use for control in the boundary group of patients, and the pair of prognostic markers TK/LDH can use for control after period of treatment.

Table 1. Correlation between ZAP-70 level and others prognostics markers in B-CLL patients.

ZAP-70 expression	ZAP-70/CD38			TK/LDH			CD38/B2 mg		
	Pre treatment group	After 6 month group	After 12 month group	Pre treatme nt group	After 6 month group	After 12 month group	Pre treatme nt group	After 6 month group	After 12 month group
0-10%	R=0.309 P=0.039		R=0.375 P=0.025			R=0.481 P=0.003		R=0.458 P=0.003	
10-20%	R=0.551 P=0.000	R=0.475 P=0.005	R=0.465 P=0.000			R=0.586 P=0.001			
>20%	R=0.469 P=0.004	R=0.671 P=0.000				R=0.451 P=0.012			

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ANALYSIS OF BLOOD LYMPHOCYTOSIS MIMICKING OF ADULT CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. In most cases, the presence of an absolute lymphocytosis and lasting (more than 3 months) in adults is related to the existence of a B-chron-

ic lymphoproliferative disorder (B-CLD). It is important to characterize the exact type of CLD, because the therapeutic management varies with the blood disease. Lymphocytosis is a benign event frequently observed after conditions of stress or a viral infection. Sustainable lymphocytosis was also described in rheumatoid arthritis and Hodgkin's disease. The blood smear examination is the first step of diagnosis. Morphological analysis must be completed within a second time by the study of lymphoid cells by flow cytometry (FCM) to distinguish malignant proliferations B or T, and reactive lymphocytosis. **Methods.** 66 patients with blood lymphocytosis have been included in this study for two years [January 2010-December 2011]. We performed a systematic way a complete blood count, a very careful cytological analysis of blood smears stained with MGG, and immunophenotypic study of lymphoid cells in peripheral blood by CMF. In some cases the comparison with histology (bone marrow biopsy) was necessary. **Results.** The average age of the study population was 61. 45 years [20-90], with a male predominance (53 men 13 women) and a sex ratio = 4. 07. At the NFS, the average WBC = 55,711 élts/mm3 [1840-436 490], with an average rate of lymphocytes élts/mm3 = 47 642 [1380 to 405 935], a mean Hb = 11,04 g/ dl [5. 7 to 14. 9], and an average of 154 288 platelets élts/mm3 [13 000-446 000]. The myelogram was practiced in 13 cases (8%), and bone marrow biopsy was performed in 15 cases (10%). In CLL (n = 37 cases), there is a very interesting way to FS, a monomorphic appearance, with the constant presence of non-slotted small mature lymphocytes and shadows of Gumprecht. The score for Matutes (SM): = 3 (n = 7 cases), = 4 (n = 15 cases), and = 5 (n = 15 cases). During the B-CLD (n = 16): the cytological aspect depends on the type of lymphoma. Two cases of hairy cell leukemia, with a score of hairy cell (CD11c, CD25, CD103) = 3. One case of follicular NHL, with a SM = 3. Six cases of MCL, with SM <3. Four cases of MZL, with SM <3 (n = 3) = 3 (n = 1). A case of SLVL with SM = 1. Two cases of small cell B-NHL difficult to classify, with SM <3. The T-CLD (n = 3): 2 cases of leukaemia NHL-T and one case of Sézary syndrome. There 09 cases of benign reactive lymphocytosis, and 01 cases of CMML, with a Matutes score = 1 (CD5 +) and an aspect polyclonal B-cell CD19 + (expression of two light chains). **Conclusions.** The lymphocytosis is frequently encountered when reading in the blood. On a blood smear, the reactive nature of the lymphocytosis has not escaped the eye of a hematologist warned. A persistent lymphocytosis should be investigated, including immunophenotypic analysis by FCM. Admittedly, there is always a risk of missing a CLD B or T.

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VALUE OF SERUM THYMIDINE KINASE LEVEL, EXPRESSION OF ZAP-70 PROTEIN AND CD38 ANTIGEN IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA WITH STAGE A

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Background. The clinical course of chronic lymphocytic leukemia (CLL) is characterized by extreme heterogeneity. We know a lot of factors to evaluate its prognosis. The most significant of these is the mutation status of the heavy chain variable regions immunoglobulin genes (IgVH). However, using this method in clinical practice is very difficult. At the same time the expression of protein ZAP-70 and antigen CD38 is considered to be a reflection of mutation status IgVH. Along with surrogate markers in recent years, much attention is paid to the study of cell proliferative activity of CLL, for which the enzyme thymidine kinase (TK) may serve as an indicator. **Aims.** To determine the clinical course of CLL, depending on the content of TK, expression of ZAP-70 and CD38 at the time of diagnosis. **Methods.** Ninety-eight CLL patients aged 35 to 79 years (median 62) were included in the study. All patients were in Binet stage A. At the time of diagnosis the expression level of Zap-70 and CD38 by flow cytometry, has been investigated, the threshold of positive expression of ZAP-70 was 20%, CD38 - 30%. The content of serum TK was assessed by a radioenzyme method. The level of TK in healthy people (n=50) was equal to 5. 2 U/L (95% CI: 4. 3-6. 8). Patients were divided into 2 groups by the nature of the flow of CLL. The first group included 67 (68%) patients with indolent disease course, in which the median treatment free survival (TFS) was 36 months. The second group included 31 (32%) patients with progressive CLL, in which the median TFS was 8. 7 months. The formation of groups was performed retrospectively. The observation of the course of disease was three years or more in every patient from the moment of diagnosis. **Results.** A positive expression of ZAP-70 was observed in 21 (31%) patients with indolent CLL, CD38 in 16 (24%). In the group with progressive disease the expression of ZAP-70 was positive in 30 (97%) patients, CD38 in 27 (87%), so it is almost 3 times higher than in patients with the sluggish variant of CLL. The content of the TK was 22. 6 U/L (95% CI: 20. 5-26. 3) in the progressive form of the disease. Whereas in the indolent form of CLL TK level was equal to 12. 7 U/L (95% CI: 8. 3-14. 9). At the same time the correlation between the expression of ZAP-70, CD38, and TK levels in patients with indolent CLL was not found (R=0. 199; p=0. 137 and R=0. 163; p=0. 437, respectively). **Conclusions.** The positive expression of ZAP-70, CD38 correlates with progressive CLL, but this relationship is not absolute. Along with the expression of ZAP-70, CD38 the determination of serum TK at the time of diagnosis of CLL adds prognostic information. For a more precise stratification of the patients a complex of prognostic factors, including the ZAP-70, CD38, and TK should be used on the risk of disease progression at the same time, which allows to identify a subgroup with a poor prognosis among patients with indolent course of CLL.

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COMPLEMENTARY AND ALTERNATIVE MEDICINE USE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A MULTICENTRIC ITALIAN SURVEY

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Background. According to the National Institute of Health (NIH), Complementary and Alternative Medicine (CAM) is defined as a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine and, generally, have limited scientific evidence. The use of CAM is common in cancer patients and is steadily increasing over time. **Aims.** This study reports the preliminary results of an ongoing Italian multicentric survey in which we would like to assess the use of CAM in patients with chronic lymphocytic leukemia (CLL) and to identify social and economical features related to its use. The protocol was approved by local Ethical Committees and informed consent was given by patients. **Methods.** Data were collected by means of a standardized questionnaire with several items assessing the use of CAM, reasons for CAM use, as well as demographic, disease status and treatment. The sample consisted of 309 patients with CLL followed at 13 Italian Hematologic Institutions. **Results.** One hundred seventy-seven (57%) patients were male and 132 (43%) female. Mean age was 69 years (range 37-94 years). The majority of patients came from Southern Italy (59%), the remaining patients came from Central (32%) and Northern Italy (9%), respectively. Of 309 participants, 42 (14%) were found to be CAM users. The majority of them started CAM after the diagnosis of CLL. The most commonly CAM therapies were green tea (18 patients), aloe (7 patients), and high dose vitamins (7 patients). Main sources of information about CAM were friends, family, media, physicians and Internet. Predictors for CAM use were a higher education level (p <0. 01) and internet availability (p <0. 001). No difference, however, were found among gender, age, occupation and urban or rural area of residence. **Conclusions.** CAM is frequently used in CLL patients. The reasons for CAM popularity among these patients are complex. Given the number of patients combining conventional therapy with CAM and the possible drug interactions, doctor interest as well as patient education about CAM should be improved.

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FRONT-LINE THERAPY WITH CHLORAMBUCIL PLUS RITUXIMAB IN ELDERLY AND/OR UNFIT PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Despite the increasing use of combination therapy with rituximab (R), fludarabine and cyclophosphamide for chronic lymphocytic leukemia

(CLL), a significant proportion of patients (pts) are not suitable for such intensive chemotherapy regimen because of co-morbidities and/or age. In those pts unfit for fludarabine based regimen, chlorambucil (CHL) remains a widely used first-line therapy. Its combination with monoclonal antibodies such as R seems to increase the overall responses rates (ORR) with good tolerability. Up to now two cooperative studies showed an ORR of 82% with a progression free survival (PFS) of 23,5 months after association of CHL-R as induction therapy for untreated elderly CLL patients. **Aims.** The purpose of this study was to evaluate the safety and efficacy of CHL-R as front-line therapy in elderly pts and /or unfit to Fludarabine regimens. Secondary endpoints included the adverse event profile, PFS, time to re-treatment and overall survival. **Methods.** At our institution, starting from February 2008, 22 pts(14 male, 8 female) received CHL (1 mg/kg p. o. at standard dose of 10 mg/day every 28 days for 8 cycles) plus R (day 1 375 mg/m^2 i. v. cycle 1, 500 mg/m^2 cycles 2-6) as front-line therapy. At study entry FISH analysis, IgVH mutational status, expression of Zap-70 and CD38 were evaluated. Fourteen pts were elderly and 8 unfit for CIRS; median age was 70 years (range 58-81). Four pts were in Binet stage A, 10 in stage B and 8 in stage C. Biological profile was characterized by good FISH profile (del13q, normal karyotype, trisomy 12 and del11q in 11, 6, 2,3 pts respectively). Moreover 7 pts were CD38+ and 6 pts were Zap-70+. Eighteen pts were informative for IgVH mutational status and 11 pts were unmutated. **Results.** The ORR was 73%; seven pts (32%) obtained CR and 9 pts PR (41%). A progressive disease (PD) was recorded in 1 patient and a stable disease in 3 pts. Two out of 22 did not complete therapy for progressive disease (one patient for autoimmune haemolitic anemia and one for increasing lymphoadenopathy). Median PFS and time to treatment was not reached at a median of 16 and 14,5 months respectively. Five pts showed PD and 6 pts required therapy; 3 pts died (2 for secondary solid neoplasia and 1 patient for PD) after 17, 31 and 23 months respectively. None of 22 enrolled patients required dose reduction of chemo-immunotherapy or needed hospitalization. Five patients (23%) experienced haematological toxicity (4 pts with grade IV neutropenia requiring G-CSF) and six patients extra-haematological toxicity consisting of tumor lysis syndrome in 5 pts and CMV reactivation in 1 patient. **Conclusions.** These data confirm that in elderly/unfit untreated CLL pts the combination of CHL-R is efficacy and well tolerated, resulting in a good ORR. Future multicentre study with a higher number of patients and with a longer follow-up will be planned to solve the question if this treatment could be useful for elderly and/or unfit patients and if a maintenance therapy could be recommended.

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CLINICAL SIGNIFICANS OF SERUM IGG SUBCLASS DEFICIENCY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Hypogammaglobulinemia is a well known immune defect in patients with Chronic Lymphocytic Leukemia (CLL). Serum levels of immunoglobulins (Ig) are routinely measured and patients with hypogammaglobulinemia and recurrent infections are offered prophylactic intravenous Ig substitution. The occurrence of selective IgG-subclass deficiency (with normal total Ig levels) and its clinical significance in patients with CLL is not well defined. **Aims.** To assess the prevalence of IgG subclass deficiency and its correlation to increased risk of infectious complications in patients with CLL. To investigate the clinical value of routine measurement of serum IgG subclass levels. **Methods.** At a given point year 2011, patients with the diagnosis CLL at Uppsala University Hospital, diagnosed 1987-2011, were asked to participate in the study. Serum levels of Ig and IgG subclasses were determined. Hypogammaglobulinemia was defined as: IgG < 6,7 g/L, IgA < 0,88 g/L and IgM < 0,27 g/L. Subclass deficiency was defined as IgG1 < 2,8 g/L, IgG2 < 1,15 g/L, IgG3 < 0,24 g/L. Clinical data regarding patient and disease characteristics were collected through patient records and questionnaires. Possible risk factors for infections and Ig-defects were analyzed. **Results.** A total of 111 CLL patients, median age of 71 (range 49-90), were included. The median disease duration at inclusion was 48 months (range 4-286), and 33. 3% had previously received chemotherapy. The annual overall risk for simple (outpatient) infections, severe (inpatient) infections and sepsis were 79. 3%, 12. 6% and 2. 3% respectively. Hypogammaglobulinemia was common and occurred in 52. 3% of the patients. Those with hypogammaglobulinemia had longer disease duration (median 61. 5, range 5-286 vs. 35, range 4-222 months, P=0. 004) and had more frequently received treatment for CLL (44. 8 vs. 20. 8%, P=0. 009), than patients without hypogammaglobulinemia. However, patients with hypogammaglobulinemia did not have a higher annual risk of infections than patients without hypogammaglobulinemia (79. 5 vs. 79. 1%, P= 0,706 for all infections; 13. 4% vs. 11. 2%, P= 0. 394 for severe infections and 1. 7% vs. 3. 4%, P= 0. 083 for sepsis). Selective subclass deficiency was uncommon and occurred in six of 111 patients (5. 4%). Although the figures were small, the annual overall risk of infections, of severe infections or of sepsis for these patients did not differ from patients with no hypogammaglobulinemi and no subclass deficiency (70. 8 vs. 80. 7%, P=0. 334, 11. 8 vs. 11. 1%, P=0. 497 and 8. 9 vs. 2. 3%, P=0. 067, respectively). **Summary and Conclusions.** Selective IgG subclass deficiency is rare in patients with CLL. In this heterogeneous cohort of patients, neither hypogammaglobulinemia nor selective IgG subclass deficiency were significant risk factors for infectious complications. Measurement of serum levels of Ig may be motivated in selected patients with recurrent severe infections, but routine analysis of IgG subclass levels in patients with CLL is not warranted.

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DETECTION OF MINIMAL RESIDUAL DISEASE IN PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA USING BOTH FOUR-COLOR AND EIGHT-COLOR FLOW CYTOMETRY AND QUANTITATIVE REAL TIME PCR APPROACH

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Background. Minimal residual disease (MRD) is a routinely used term for small population of pathologic cells that remain during or after treatment when the patient is in remission. Rawstron et al. (2007) published four-color flow cytometric method, which made possible to standardize MRD assessment in patients with chronic lymphocytic leukemia (CLL). Application of this method enables to determine the MRD level in peripheral blood or bone marrow down to less then 0. 01% of leucocytes. The results can be compared to RQ-ASO IgH PCR (real-time quantitative allele-specific oligonucleotide Immunoglobulin Heavy chain gene PCR). In ASO-PCR individual patient-specific oligonucleotide primer are designed to detect MRD, its sensitivity reaches to 1 cell in 10^6. **Aims.** The aim of our study was to compare multiparametric flow cytometry to molecular biology approaches with detection of IgVH specific clones in patients with CLL diagnosis. **Methods.** Cohort of 22 CLL patients who underwent allogeneic stem-cell transplantation (SCT) was included in the study. MRD were analyzed employing four-color flow cytometry protocol (Rawstron, 2007) together with eight-color flow cytometry protocol. Cell surface were stained with fluorescence labeled monoclonal antibodies (anti-CD3, 5, 14, 19, 20, 22, 38, 43, 45, 79b and 81). Flow cytometric acquisition was performed on a FACSCantoII flow cytometer (Becton Dickinson,NJ,USA). Detection of clonal IgVH was performed by RQ-PCR with LNA probe (Locked Nucleic Acid, TaqMan technology). **Results.** Since RQ-ASO IgH PCR shows sensitivity attaining 1 cell in 10^6, 8. 6% of samples were found positive using RQ-PCR but negative using flow cytometry. However, 10 patients were simultaneously observed using flow cytometry and RQ-PCR method with strong correlation (r=0. 94). In 17 patients both flow cytometric protocols (four- and eight-color) were employed to observe the correlation of these two methods. It was found out that the correlation coefficient is getting closed to one (r=0. 96). **Summary and Conclusions.** We compared multiparametric flow cytometry to molecular biology approaches with detection of IgVH specific clones in patients with CLL diagnosis. Despite lower sensitiveness, flow cytometry is adequate method to MRD detection. Concurrently, we demonstrate potential application of eight-color flow cytometric protocol. The advantage of this protocol lies in simultaneous detection of more than two markers compared to four-color cytometry. **Acknowledgement.**This work was supported by grant MUNI/A/0784/201.

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CD-MORPHOLOGY MICROARRAY - A NEW DIAGNOSTIC METHOD IN ONCOHEMATOLOGY APPLIED TO HAIRY CELL LEUKEMIA

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Background. Current diagnosis of oncohematological malignancies is based on a variety of methods. Most widely used are the blood or bone marrow smear examination for leukocyte morphology and immunophenotyping. The presence of leukocytes with atypical or pathological morphology in smears is

in many cases the first indication of hematological neoplasia. At the same time, in case of a suspected lymphoproliferative disorder the final diagnosis requires the differentiation between the lymphocyte subpopulations that cannot be done by morphology alone. The flow cytometry, currently used for immunophenotyping, allows to define the general amount of cells with a specific immunophenotype but gives no information about the cell morphology. **Aims.** The impossibility of applying both methods to the same cell can lead to controversies in diagnosis. We have developed a new diagnostic method for simultaneous determination of CD markers and complete cell morphology analysis based on a CD-Morphology microarray. **Methods.** CD-Morphology microarray is a set of monoclonal antibodies specific for 35 leukocyte surface antigens including isotype controls spotted on a transparent plastic slide. Upon incubation of leukocyte suspension with the microarray, cells with specific surface antigens are binding to corresponding immobilized antibodies. After the nonspecifically bound cells are washed away, areas of leukocytes positive for individual CD antigens stay bound with the microarray. The morphology of the bound cells can then be studied after standard cytological staining. Thus the CD-Morphology microarray allows to investigate the morphology of blood cells for which one of its surface CD antigens is known. **Results.** We have studied lymphocyte suspensions from peripheral blood of 10 patients with suspected hairy cell leukemia. Hairy cell leukemia (HCL) is a mature B-cell lymphoproliferative disorder characterised by distinct morphology and immunophenotype. HCL is diagnosed based on the presence of "hairy"or villous lymphocytes with strong expression of CD19, CD20, CD22, CD11c and moderate expression of CD103 and CD25 in blood or bone marrow smears. In all studied patients immunophenotype and lymphocyte morphology in smears corresponded to HCL. In 7 patients we have found lymphocytes with cytoplasm edge irregularities ranging from hairy projections through shaggy to serrated or festooned borders positive for CD11c, CD19, CD20, CD25 and CD103 that permitted us to suggest the presence of hairy cell leukemia. In two of those seven patients the hairy cells (HC) were also positive for CD2. For this patient group the HCL diagnosis was confirmed by histological and cytochemical examination. The peripheral blood of the remaining 3 patients contained small amount of CD11c+ lymphocytes, but no CD103-positive lymphocytes. In all of them various amounts of villous lymphocytes positive for CD19 (1-10% of total B-cells) but not for 11c were detected. We have also detected a group of CD19+, CD20+, CD22+ lymphocytes uncharacteristic for HCL and morphologically similar to neoplastic cells seen in patients with splenic marginal zone lymphoma (SMZL). These patients were diagnosed with SMZL based on immunophenotyping, histology and cytochemistry. **Conclusions.** The CD-Morphology microarray can be used in differential diagnostics of hairy cell leukemia vs splenic marginal zone lymphoma with villous lymphocytes.

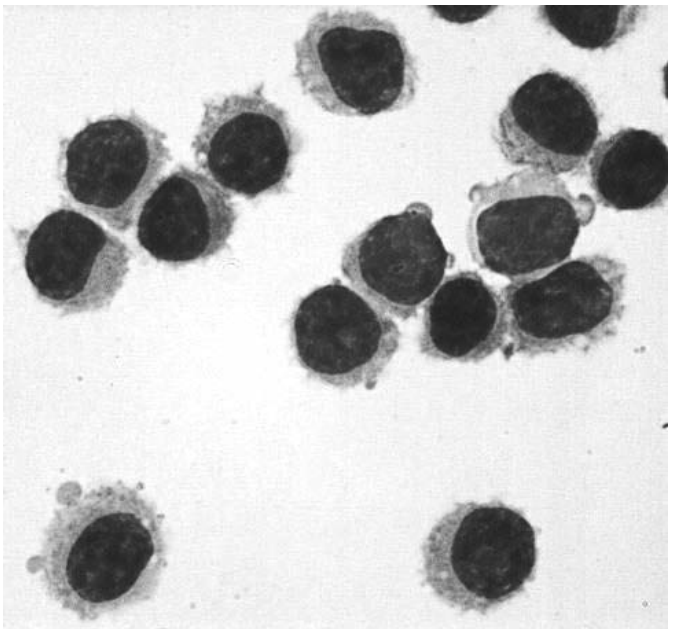

Figure 1. The morphology of CD11c+ lymphocytes isolated from peripheral blood of a patient with hairy cell leukemia.

1293

RITUXIMAB INFUSION-RELATED TOXICITY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background and Aims. Rituximab in combination with chemotherapy is an effective treatment of patients (pts) with chronic lymphocytic leukemia (CLL). The most frequent adverse event of rituximab is infusion-related toxicity, e. g. cytokine-release syndrome that occurs usually during the first infusion. However, there is scarce data on feasibility and tolerability of rituximab infusions in CLL outside clinical trials. Therefore, we performed a single-center retrospective analysis of the frequency of rituximab infusion-related adverse events during the first- and the second line CLL treatment administered in the routine practice. We also analyzed its relation to parameters of tumor load and possible association with treatment efficacy. **Patients and Methods.** We analyzed 88 pts (62 males, median age, 62 years [range, 31-84]) with CLL treated between March 2005 and May 2011 at our institution. The most common first-line regimens were FCR (37 pts) and low-dose FCR (13 pts); 5 pts were treated by other protocols. Thirty-three pts underwent second line treatment: 7 pts FCR, 12 pts rituximab-dexamethasone, 6 pts low-dose FCR and 8 pts other regimens. Intravenous hydration (2000 ml on days 0 and 1 of cycle), allopurinol 300-600 mg p. o. and premedication with methylprednisolone 80 mg i. v., acetaminophen 1000 mg p. o. and bisulepine 1 mg i. v. were administered before rituximab infusion. Rituximab was given by fractionated infusion (100 mg for 2 hours, then if tolerated well, the rest of the dose with infusion rate escalation from 100mg/hour up to 400mg/hour) in a total dose 375 mg/m^2 in the first cycle and 500 mg/m^2 in subsequent cycles. **Results.** Rituximab infusion-related toxicity occurred in 35% pts (n=19) during the first line treatment and 21% pts (n=7) during the second line treatment. Adverse events were predominantly mild and NCI CTCAE grade III/IV occurred rarely (4% in the first line, 3% in the second line). Infusion toxicity manifested predominantly as rigors, chills, fever and/or hypotension and grade III/IV adverse events were syncope, respiratory distress and hypotension with collapse. All patients with adverse events could finish rituximab infusion at initially planned dose on the same day. Treatment response analysis did not demonstrate statistically significant differences between patients with and without rituximab infusion toxicity (fist line ORR 86 vs. 89%, second line 86 vs. 68%). Patients who developed rituximab infusion toxicity had higher initial absolute lymphocyte count (first line, 87 vs. 56 x 10^9/l, p=0. 21; second line, 101 vs. 14 x 10^9/l, p=0. 043). At the median time of follow up 27. 7 months, there were no statistically significant differences in PFS or OS in both cohorts. **Conclusions.** Rituximab infusion-related toxicity in pts with CLL is relatively frequent (35%). However, occurrence of infusion-related symptoms can be reduced by proper premedication and severe adverse events are uncommon. In our experience, all patients were able to receive the planned dose of rituximab. We did not find statistically significant association between rituximab infusion toxicity and effectiveness of treatment. Acknowledgements: Supported by research project MZO 00179906 from Ministry ofHealth,Czech Republic.

1294

DOSE DENSE HIGH DOSE METHYLPREDNISOLONE (HDMP) AND RITUXIMAB (RTX) ARE EFFECTIVE IN RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH 17P DELETION (17P DEL) / TP53 MUTATION

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Background. Management of CLL patients with defective *TP53* pathway is a considerable challenge and the prognosis remains dismal. There are a few treatment options, including monoclonal antibody alemtuzumab as monotherapy or in combination, but response rates, progression free survival (PFS) and overall survival (OS) are still unsatisfactory. **Aims.** was to evaluate the efficacy and safety of dose dense HDMP and Rtx combination in relapsed CLL patients with 17pdel/*TP53* mutation in a frame of LT-CLL-001 study (described earlier[1]). **Methods.** Patients with 17p del/*TP53* mutation, who had relapsed or progressive disease after at least one line of chemotherapy were treated. HDMP was administered at 1 g/m^2 intravenously daily for five consecutive days of each treatment course. Rtx was administered at a dose of 375 mg/m^2 on day 1 and 500 mg/m^2 on day 5 of the first course, on days one and five of the second course, and on day one of courses three to six. Courses were repeated every 21 day for a total of 6. Response was evaluated according NCIWG-96 criteria. **Results.** 14 patients (13 enroled into a prospective study[1] and 1 on compassionate use grounds) were treated. Median age was 62 years (range

46 - 76), 11 (79%) patients had Rai III-IV stage, 8 (57%) had bulky (> 5 cm) lymphadenopathy. Five (36%) had 17p del only, one (7%) - *TP53* mutation only and 11q deletion, 8 (57%) patients had 17p del and *TP53* mutation, 4 (29%) patients were fludarabine refractory. Median number of HDMP-Rtx courses was 6 (1 - 6). Overall response rate (ORR) was 72%, all partial responses (PR), one patient (7%) had stable disease, 3 (21%) patients progressed. Two patients with PR underwent allogeneic bone marrow transplantation and are alive and free of disease to date. After the median follow-up of 30. 5 months, the median PFS is 12 months (range 9 - 15) and median OS is 31 month (26 - 36) (Figure 1). The most common toxicity was hyperglycemia not requiring intervention. 4 cases of III-IVº neutropenia and 2 cases of febrile neutropenia were observed. There were 3 (one treatment related) early deaths. **Conclusions** The dose dense HDMP and Rtx is effective and has acceptable safety profile in pretreated CLL patients with 17p del/*TP53* mutation. However it should be evaluated in a larger cohort as first or second line therapy, also before allogeneic bone marrow transplantation.

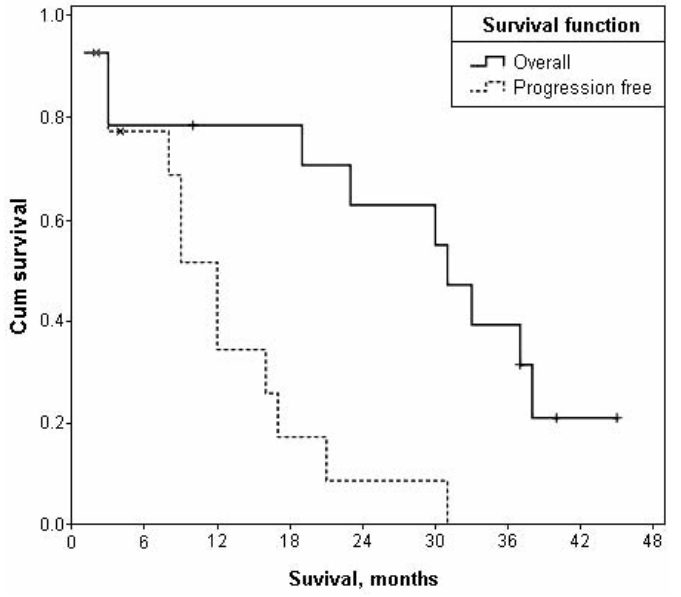

Figure 1. PFS and OS.

References

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1295

MATURE B-CELL NEOPLASMS AND RENAL CELL CARCINOMA OF CLEAR CELL TYPE - IS THERE A LINK?

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Background. Mature B-cell malignancies (i. e. B -lymphoproliferative diseases, LPD) and renal cell carcinoma (RCC) account about 4% and 3% of all adult malignancies, respectively. Besides the trend of steady increase in incidence of both malignancies**,** several population-based epidemiological studies have also confirmed previously reported clinical observations of higher than expected occurrence of both malignancies in a same patient. It is shown that patients with RCC have 1. 51 and 1. 86 higher overall relative risk of multiple myeloma (MM) and non-Hodgkin lymphoma (NHL) than general population, respectively. On the other hand, the relative risk for developing RCC in patients with MM is 1. 89 and in NHL patients is 2. 67**.** Several reasons for that such as primary cytotoxic treatment, interactions of several environmental factors, genetic predisposition, viral infections or immunomodulatory effects of tumor itself are postulated, but the etiology is not jet clarified. **Aims.** To evaluate demographic, clinico- pathological characteristic and outcome of patients with LPD and RCC co-occurrence. **Methods.** Medical records of 680 consecutive patients with LPD and 570 consecutive patients with RCC diagnozed between January 1997 and December 2011 were retrospectively analyzed. **Results.** We found co-occurrence of both diseases in five patients (2. 5%; 3 males and 2 females) whose median age at LPD diagnosis was 58. 6 years (range 44-70). In two patients LPD were diagnosed synchronously with RCC. Indolent chronic lymphocytic leukemia (CLL) preceded RCC in one patent, while advanced-stage MM refractory to applied chemotherapy was diagnosed after RCC in two patients after a very short latent period. According to RCC type, all five patients had localized clear-cell RCC, predominantly of right kidney. None of these patient s have received any adjuvant therapy after nephrectomy and there was no sign of RCC recurrence during their last follow-up (Table 1). **Conclusions.** Although the number of patients in our study is small, our results suggest the significant association of LPD and clear-cell RCC. Extranodal presentation of NHL and poor outcome of patients with MM are in line with previously reported data. Synchronous occurrence of LPD and RCC or short latent period between the diagnoses of these two malignancies in the same patient, as well as no cytotoxic treatment for firstly occurring neoplasm indicate the common pathobiology of both diseases.

Table 1. Summary demographic, clinical and pathological characteristics.

No	Sex	Age at RCC	Type and TNM stage of RCC	RCC Th	LP	Age at LPD	Type and CS of LPD	LPD Th	Outcome & Survival
1	M	69	Clear cell type pT1N0M0	right N	8	70	MM IgA kappa IIIB	VAD MPT	17 months
2	M	44	Clear cell type pT2N0M0	right N	0	44	NHL DBCL IE /oral cavity/	CHOP	CR ≥ 7 years
3	F	68	Clear cell type pT2N0M0	left N	0	68	CLL A	Ø	alive ≥ 15 years
4	F	46	Clear cell type pT2N0M0	right N	18	48	MM IgG kappa IIIB	VAD MP	25 months
5	M	64	Clear cell type pT1N0M0	right N	-28	60	CLL A	Ø	alive ≥ 5 years

Legend: Th-treatment, N-nephrectomy, LP-latent period(months), CS-clinicalstage

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KIDNEY INVOLVEMENT IN CHRONIC B-CELL LYMPHOCYTIC LEUKEMIA

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Background. Patients with non-Hodgkin lymphoma can develop renal failure in many ways: obstruction, infiltration of various parts of urinary tract. This can lead to renal failure. And finally, kidneys can be damaged as a result of treatment. Leukemic infiltration of the kidney has been found in over 60%-80% of the autopsies performed patients with chronic lymphocytic leukemia (CLL), but it is only rarely associated with renal failure. **Aims.** of this study To determine the incidence of kidney damage in patients with chronic lymphocytic leukemia and identify the morphological features of kidney damage in patients with CLL, complicated by the development of acute renal failure. **Materials and Methods.** Following retrospective analysis, 30 cases with CLL from the Department of Pathology of the Hematological Research Center(Moscow, Russia) were examined by light microscopy. All patients were treated with various regimens of chemotherapy and undergoing to autopsy between January 2000 and December 2010. We reviewed kidney specimens and assessed them for the presence of leukemic infiltrates. There were 24 male and 6 female. The median age was 61 years (from 43 to 71 years). Patients over 60 years were 13. Disease duration was from 3 to 64 months. All patients were died in the intensive care unit in connection with the development of complications during chemotherapy. Acute renal failure (ARF) was observed in 18 (60%) patients and in same patients hypertension were in 3 cases, diabetes mellitus - in 2 cases, hyperuricemia - in 4 cases, paraprotein secretion - in 5 cases, hyperleukocytosis (45 - 219 x 10^9/l) in peripheral blood - in 11 cases. Acute tubular necrosis in histological specimens was revealed in cases of CLL with acute renal failure. **Results.** Morphological study of histological specimens of autopsied kidney was detected leukemic infiltration in 26 (86. 7%) cases. Leukemic infiltration of the glomeruli was detected in 11 (42. 3%) cases, while in other cases infiltration only interstitial. Leukemic infiltration of the glomeruli was detected in cases where CLL was characterized with hyperleukocytosis. In cases with secretion of paraprotein there were morphological signs of glomerulitis. Sclerosis and fibrosis was apparent in cases with concomitant diseases (hypertension, diabetes mellitus). **Conclusions.** Thus, more than in 80% cases renal

damage was observed in CLL according to the autopsy. The development of acute renal failure not only related to the therapy or complications or concomitant disease, but also associated with clinical manifestations of the disease (hyperleukocytosis, M-component). The massive leukemic lesions of the glomerular apparatus were observed only in patients with hyperleukocytosis.

1297

COMBINATION OF THE DISTINCT CLINICAL AND BIOLOGICAL MARKERS OF CLL PATIENTS IN THEIR PROGNOSTIC STRATIFICATION: PRELIMINARY RESULTS FROM SINGLE CENTER EXPERIENCE

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Introduction. The clinical course for patients with chronic lymphocytic leukemia is extremely heterogeneous, some patients have indolent disease, never needing treatment, whereas others have aggressive disease requiring early treatment. One of the most important challenges in the clinical management of those patients is the decision of initiating their treatment because there is no available prognostic system that will resolve this issue. Usually, criteria for active disease are used to initiate therapy. Recently, some authors proposed prognostic models that combines a set of clinical risk factors and estimates individual patient survivals. Here, we report our initial results from a study designed to evaluate the statistical association of the distinct clinical and biological parameters with the prognosis and time to initiating treatment for patients with CLL. **Materials and Methods.** Our study incorporated 115consecutive, treatment naïve CLL patient. In each patient all traditional laboratory, clinical and biological prognostic factors were evaluated at their first visit to our Institution. Than we combined the following independent characteristics: age, β-2 microglobulin, absolute lymphocyte count, sex, Rai stage, and number of involved lymph node groups, which are included in some of the already published CLL prognostics index, in association with the CD38 expression. Further, we correlate those factors by multivariable analysis with time to first treatment. This multivariable model was used to develop a nomogram-a weighted tool to calculate 5- and 10-year survival probability a 2- and 4-year probability of treatment and estimate median time to first treatment. **Results.** According to prognostic index a classification tree was built that identified three subsets of patients who scores were 1-3 (low risk- 20pts-17,3%), 4-7 (intermediate risk-80pts-69,5%) and >8 (high risk-15pts-13%). Estimated median survival at low risk subset of patients is 22,5years, 10,8 and 4years respectively at intermediate and high risk subsets of patients. Projected survival in respectively low, intermediate and high-risk groups was 70%, 92,5%, 100%, and 100%, 99%, 60% at 5-year and10-year, respectively. The following were independently associated with shorter time to first treatment: Also, statistical analyses showed that three involved lymph node sites and CD38 expression is associated with shorter time to first treatment. **Conclusions.** Our prognostic model that combines and correlates the distinct clinical and biological markers of CLL patients enables identification of the patients that are at high risk for progression. This prognostic model may facilitate clinical decision for initiating treatment. Reply to: Send

1298

THE HISTORY OF CHRONIC LYMPHOCYTIC LEUKEMIA IN A SINGLE CENTRE OF HEMATOLOGY: CLINICAL AND BIOLOGICAL CHARACTERISTICS AND OUTCOME IN 315 PATIENTS AFFECTED BY CLL DIAGNOSED DURING THE LAST 11 YEARS

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Background. Chronic Lymphocytic Leukemia (CLL) is the most common lymphoproliferative disorder of the elderly population in Western countries. Clinicians collect many clinical and biological data useful for prognosis and management of the disease. No comprehensive data on epidemiological, clinical and biological characteristics and evolution of the B-CLL population are available in a single centre analysis. **Aims.** This retrospective study has the purpose to analyse demographic, clinical and biological characteristics of our B-CLL population, diagnosed and followed at Institute of Hematology of Catholic University of Rome. **Methods.** From our database consisting of 402 diagnoses during the last 11 years, we excluded 87 patients previously treated in other centre or referred to our centre for consultancy. Thus we selected 315 patients affected by CLL diagnosed from January 2000 to December 2010 and followed until May 2011. The diagnosis of CLL was made according to criteria from the National Cancer Institute. For each patient in the database we reported clinical and biological features at diagnosis, comorbidities, any relevant clinical condition developing during CLL history, progression free survival (PFS), time to treatment (TTT), overall survival and cause of death. **Results.** We recorded a mean of 29 B-CLL diagnosis per year (range 14-41). The median age at diagnosis was 67 years (range 35-89 years) with a ratio M:F of 1. 6:1 (195 males, 120 females). The Binet stage was A stage in 228 patients (72. 4%), B stage in 64 (20. 3%) and C stage in 23 (7. 3%). The median value of absolute lymphocyte count was 10. 025 lymphocytes/mmc (range 1000 to 240. 000), 45 patients (14. 3%) were diagnosed as Monoclonal B lymphocytosis because of clonal lymphocytes were less of 5000/mmc. Biological characteristics consisted of positivity of ZAP-70 in 108 pts (36. 9%; data available for 293 pts), CD38 in 71 pts (24. 1%; data available for 295 pts) and CD49d in 56 pts (42. 7%; data available for 131 pts). The analysis of chromosomal aberrations conducted in 279 patients by FISH demonstrated del(13q14) in 72 pts (25. 81% of the investigated pts), trisomy 12 in 44 pts (15. 77%), del(11q22) in 23 pts(8. 24%) and del(17p13) in 21 pts (7. 53%). The analysis of the IgVH mutational status in 246 available patients displayed 101 pts (41. 1%) unmutated and 145 pts (58. 9%) mutated. Analysing the outcomes the median PFS and TTT were not achieved after a median of 34. 5 and 36 months respectively. We noticed 153 progressions (48. 6% of the pts) after a median time of 25 months (range 1-132) and 132 pts (41. 9%) were treated after a median time of 24 months (range 1-109). After a median of 56 months (range 5-136 months), 15 patients were lost at follow-up and 56 patients died (17. 8%):26 pts (46. 5%) for causes related to CLL, 7 pts for neoplasia, 5 pts for heart diseases and 10 pts for unspecified extra-hematological diseases. In 8 cases, it was not possible to document the cause of death. **Conclusions.** Confronting our analysis to the literature data we found that the cohort of our patients is representative of the CLL population.

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EFFICACY, TOLERABILITY, COST-SAVING OF FRONTLINE ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINATION THERAPY FOR CHRONIC B-CELL LYMPHATIC LEUKAEMIA AND LOW GRADE NON HODGKIN LYMPHOMA ELDERLY PATIENTS

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Background. Treatment decision of elderly patients has to be made individually considering, not only stage and risk factors of the disease, but also patients' physical condition and social environment. Fludarabine was the first purine analogue with an oral formulation available for clinical use. Oral formulation offers equivalent efficacy and an improved tolerability profile compared to the intravenous (IV) formulation. IV fludarabine requires several administrations that will expose patients to the risk of IV injection complications and cost for going to hospital. **Aims.** We would like to show that frontline oral fludarabine and cyclophosphamide combination therapy, for B-cell lymphatic leukaemia and low grade non Hodgkin lymphoma aged patients, is well tolerated, efficacy and cost-saving. **Methods.** Between April 2005 and December 2011, 10 elderly untreated patients (mean age 75, range 68-86) with treatment requiring B-cell lymphatic leukaemia (according to ESMO guidelines working group) and 13 elderly indolent stage ≥3 non Hodgkin lymphoma untreated patients (mean age 75, range 59-80) received therapy with low dose of oral fludarabine (25mg/mq/die) and cyclophosphamide (150mg/mq/die) (FC) both from days 1 to 3. Study design consisted of 6 cycles repeated at 4 weeks intervals in outpatient regimen. Patients received antibiotic prophylaxis with trimethoprim/sulphamethoxazole (160/800 mg twice a day, 3 times week) and allopurinole (300 mg once a day from days 0 to 4). Performance status was WHO £2 inall patients. Comorbidities, including diabetes, hypertension, chronic heart disease and chronic renal failure, were present in 13 patients. The mean of administered cycles was 3 with range 2-6. No patients reduced dose and number of cycles because of haematologic and extra-haematologic toxicities. Specifically only 2 patients experienced grade III neutropenia, treated with GCSF. **Results.** Definition of response was reviewed according to the updated IWCLL-NCI 2008 international general practice criteria. 20 of 23 patients (9 RC and 11 RP) obtained a response with overall response 87%. Only one responder patient died due to stroke and another patient lost response, but she didn't require therapy; mean overall survival was 28 months (range 3-56). We used Genzyme sponsored Excel program to compare direct hospital cost of oral and IV FC (both 3 days regimen). IV treatment required 18 day hospital accesses with total cost of € 7. 527, oral regimen required 6 ambulatory accesses with total cost of € 1. 642 (costs including: pharmacy, nurses and physicians resources). In this analysis we didn't consider social and psychological cost: transports, relatives' lost of working hours, disease consciousness, trauma of repeated venipunctures. **Conclusions.** These results suggest that this regimen could be effective and well tolerated for elderly patients unfit. Moreover this therapy compared to chlorambucil, the most used agent in these patients, is more effective and better tolerated. In fact although some patients relapsed or pro-

gressed, most of them do not experience severe toxic side effects or required hospitalisations, obtaining satisfactory quality of life and survival. In addition ambulatory regimen is preferred by our patients, who are treated in a friendly environment, with fewer complications and minor use of hospital resources.

1300

THE STUDY OF DYSLIPIDEMIA AND MEAN PLATELET VOLUME IN RELATION WITH THE AGGRESSIVENESS OF DISEASE, RESPONSE TO TREATMENT AND THROMBOTIC RISK IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Leukemic cells have increased cholesterol metabolism, which they capture intracellular and use it for their proliferation, which could be reflected by hypocholesterolemia. An increased mean platelet volume is an index of platelet activation and of thrombotic risk, as well as hypercholesterolemia. **Aims.** We aimed to analyze the changes induced by chemotherapy in patients with CLL on lipids and platelets. **Materials and Methods.** We studied all 64 patients with CLL in 2011, who were in the electronic filing system of the Department of Hematology of Sibiu. They were divided into 2 groups: A - who had chemotherapy during 2011 and B - without chemotherapy in 2011. We noted: gender, age, disease stage, platelet count and associated diseases. In group A patients, we have also studied the number and type of chemotherapy session and cholesterol, triglycerides and mean platelet volume before and after chemotherapy. We considered in those in group B the same biological data from their first hospitalisation in 2011. **Results.** In group A mean age was 65. 4+/-10. 4 years; the gender distribution: 8 women (42. 11%) and 11 men (57. 89%). Average disease stage: 2. 16+/-12. Cholesterol increased (p<0. 005), with an average of 30. 29+/-27. 59 mg/dl from a baseline average of 177. 44+/-49. 61 mg/dl to 186. 88+/-39. 04 mg/dl after an average of 3. 58+/-2. 04 chemo sessions. Initial cholesterol was directly correlated with the initial number of platelets (r=0. 712) and inversely correlated with disease stage before treatment (r=-0. 261), so the higher leukaemic cell mass, the lower cholesterol, which is in agreement with previous findings in literature that the leukaemic cell uses cholesterol for its proliferation. Final cholesterol was directly correlated with platelet count (r=0. 384) and inversely with patient age (r=-0. 300) and number of chemo sessions (r=0. 360). The variation of cholesterol was inversely correlated with age (r=-0. 491), with initial cholesterol (r=-0. 355) and number of chemo sessions (r=-0. 330). Triglycerides did not significantly vary, but there was a slight direct correlation between it and initial cholesterol (r=0. 264). Mean platelet volume after final chemo session was directly correlated with disease stage before chemotherapy (r=0. 626) and inversely with platelet count (r=-0. 380) and final cholesterol (r=-0. 290). In patients of group B mean age was 66. 9+/-10. 7 years; the distribution by gender: 28 women (62. 22%) and 17 men (37. 78%). Disease stage of patients in group B was significantly lower than those in group A (p<0. 00005) and correlated directly with age (r=0. 315) and inversely with cholesterol (r=-0. 255) and triglycerides (r=-0. 280). Cholesterol was not significantly different in the 2 groups, but in group B was directly correlated with serum triglycerides (r=0. 483), as in group A, but stronger. Mean platelet volume of group B was higher than that of group A post-therapy (p=0. 01) and correlated inversely with the cholesterol of group B (r=-0. 470). **Conclusions.** Cholesterol decreases as the disease stage increases and may be an indicator of mass growth of leukemia cells and of disease aggressiveness. Higher cholesterol and lower mean platelet volume after chemotherapy may be indicators to treatment response. Postchemotherapy, the decrease of mean platelet volume lowers thrombotic risk, while hypercholesterolemia rises it and requires therapeutic measures.

1301

SUCCESSFUL MANAGEMENT WITH INTRAVENOUS IMMUNOGLOBULINS IN ALEMTUZUMAB-INDUCED ACUTE INFLAMMATORY DEMYELINATING NEUROPATHY: CLINICAL REPORT OF THREE PATIENTS

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Several neurological complications have been associated with the use of monoclonal antibodies (mAbs), anddemyelinating disorders have been estimated to affect the 0. 02-0. 20% of treated patients. Alemtuzumab is ahumanized chimeric mAbthat targets the CD52 antigen, it is currently approved for relapsed/refractory and high-risk untreated chronic lymphocytic leukemia (CLL). The major complication of alemtuzumab therapy is the increased risk of opportunistic infections secondary to the profound immunosuppression. Autoimmune diseases as Graves disease, immune thrombocytopenic purpura and Good pasture syndrome, have been reported to be associated to the treatment. In the present report, we present three CLL patients developing acute inflammatory demyelinating neuropathy during treatment with alemtuzumab. Despite the severity of the complication, all the patients showed an univocal good clinical response after treatment with intravenous immunoglobulin (IVIG). As alemtuzumab represents, nowadays, a key therapeutic option for CLL, clinicians should be aware of this rare and disabling toxicity.

1302

A CASE OF REFRACTORY IMMUNE THROMBOCYTOPENIA (ITP) SUCCESSFULLY TREATED WITH ROMIPLOSTIM IN A PATIENT AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. Romiplostim is a TPO mimetic agent which has been shown to be a clinically effective treatment for idiopathic ITP. Recently its use is being extended to secondary ITP; as 2-5% of patients affected by CLL develops ITP, this agent is raising a lot of interest regarding its possible role in treatment-refractory forms. Even if some reports describe the development of bone marrow fibrosis and the tendency to venous thrombosis, adverse events are usually mild or moderate in severity; similarly there is currently no evidence to suggest that romiplostim affects the natural history of CLL. However, in both cases, more experience is required before final Conclusions can be drawn. **Aims.** We describe the case of a patient affected by CLL who develop a chronic, refractory ITP and has been successfully treated with romiplostim. **Case Report.** In August 2006, a 45-year-old man was diagnosed with CLL, stage II-Rai, A-Binet. His leukemic clone expressed unmutated IGHV and, analyzing VDJ recombination and HCDR3, he appeared to belong to stereotyped subset #7. Analyzing by FISH we reported an isolated deletion in the region 13q14. Two months from the diagnosis, the patient developed severe thrombocytopenia; a bone marrow biopsy showed normal megakaryocyte count with 60% B-lymphocyte infiltration. Diagnosis of ITP was made as all the other common causes of thrombocytopenia were ruled out. After treatment with oral prednisone (1 mg/Kg daily), platelets started increasing, so that we soon began slowly tapering the dose. However, while the patient was assuming 7. 5 mg/d of prednisone, we observed an ITP relapse; the autoimmune complication appeared to be refractory to treatment with IV immunoglobulin and with cyclophosphamide, therefore we started treatment with alemtuzumab at low doses: at the 9th week normal platelet values were reached and at the 18th also a complete response of CLL was achieved. After about two years, while the patient started again low-dose alemtuzumab because of CLL progression, platelets decreased to 9x10^9/l; bone marrow evaluation showed a scanty infiltration of B-cells and a number of megacaryocytes considerably increased. We firstly retreated the patient with prednisone and IV immunoglobulin without any efficacy; then we associated rituximab observing only a partial response on platelet values. In September 2010 ITP relapsed again and we started treatment with romiplostim 1 mcg/kg every week. Platelets soon started increasing and therapy was well tolerated. Treatment was then interrupted in February 2011 when the platelet count was permanently above 200x10^9/l. In the meantime a CT scan performed because of fever and asthenia showed pleural effusion and pleural thickening; a biopsy revealed the presence of a biphasic mesothelioma. In April 2011 the patient, still showing a normal platelet count, underwent chemotherapy without any other autoimmune complication and he is still alive on September 2011. **Summary and Conclusions.** Even if studies in a large number of patients are needed to draw definitive Conclusions, this case confirms that romiplostim could represent an useful therapeutic option for patients affected by CLL who develop chronic and refractory ITP, with prompt efficacy and few adverse events.

1303

CAN BENDAMUSTINE BE USED IN CLL PATIENTS WITH ACTIVE HEMOLYTIC ANEMIA? REPORT OF 2 CASES

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Background. Although studies have shown low prevalence of AIHA in CLL patients treated with FCR as compared to fludarabine monotherapy, safety of FCR is not sufficiently tested in patients with clinically overt hemolysis and thus FCR is rarely used in such context. Activation of AIHA usually coincides with

CLL progression and pathophysiologic links between two conditions were proposed. Immunosuppressive therapy alone is not very effective in CLL. Hence there is a need of new safe and potent agents for patients presenting with disease progression combined with severe AIHA. Beside anecdotal reports of bendamustine-associated hemolysis there is still no evidence whether this drug can be safely used in this patient group. **Methods.** Clinical and key laboratory data were collected from medical documentation of 2 relevant patients. Diagnosis and response criteria were as per IWCLL (2008). **Results.** Patient 1 was 70 yo male whose medical history comprised 5 lines of treatment (cyclophosphamide for 2 years, 6 courses of FC with PR lasting 1. 5 years, chlorambucil±prednisone for 2 years, 2 courses of fludarabine) with subsequent development of severe AIHA after F mono for which he received rituximab, prednisone and cyclophosphamide in different combinations for 2 years with relative CLL and AIHA control. He was admitted 3 mos after last rituximab dose with overt CLL progression, Rai II stage disease, fast drop in Hb level to 91 g/l, positive DAT, increased levels of reticulocytes and indirect bilirubin. Treatment with BR was initiated. Because of insufficient control of hemolysis methylprednisone (500 mg x 3 d) was added from D15. In about a week reticulocyte counts dropped to normal and Hb levels increased. Patient developed grade 2 neutropenia. Subsequent courses were R (D1) + B (D2-3) + 40 mg dexamethasone (D2-3). B dose in this combination was 55 mg/m2 initially but had to be reduced to 30 mg/m2 from course 3 because of grade 4 neutropenia and pulmonary infection. This reduced dose was well tolerated with no hematological toxicity. After course 6 CR was documented and there were no signs of hemolytic anemia (Hb 143 g/l, Ret 6 prom.). AIHA-free CR lasted for 13 mos. He had been successfully retreated with 2 courses of BRD in relapse and is currently on cyclosporine maintenance. Patient 2 was 51 yo male with AIHA already present at CLL diagnosis. His anamnesis included chlorambucil, R-CHOP, R±prednisone. At admittance he had active progression, del11q, bulky disease, Hb 61 g/l, Ret 61 prom, positive DAT and increased indirect bilirubin. He was treated with BR after methylprednisone preface (750 mg x 3 d from D-3) with additional R given on D8 and D15. After course 1 active hemolysis was eliminated and Hb levels normalized. Courses 2-5 were standard BR with PR after 3 courses achieved at the cost of transient grade 4 neutropenia and some infectious complications. **Conclusions.** In our experience combination of bendamustine with corticosteroids and rituximab is effective both in disease control and active hemolysis elimination and thus can be used even in patients with severe CLL-associated steroid-refractory AIHA.

1304

COMPLETE REMISSION CHRONIC LYMPHOCYTIC LEUKAEMIA(B-CLL) AND HEPATITIS C CONSERVED AFTER ANTIVIRAL THERAPY.

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Background. In a number of studies have shown that patients with B-CLLwith hepatitis C virus (HCV) infection makes 2-3%. Patients with B-CLL now receive more often chemotherapy with rituximab. During chemotherapy with rituximab in patients with B-CLL and hepatitis C have often develop liver dysfunction. This required antiviral therapy. **Patients and Methods.** In this research was 12 pts with B-CLL HCV RNA-positive. All 12 pts have typical immunohistochemistry (IHC) type B-CLL- CD 5+,CD20+,CD19+,CD23+. Among those patients in 2 in immunohistochemistry (IHC) analysis a expression of markers of viral protein on the tumor cells (IHC «+») were found. Median age was 62 years. The median level HCV-RNA before chemotherapy was $2x10^4$ copy/ml. At diagnosis, ALT value was above UNL in 6 patients. All pts undergoing chemotherapy with rituximab. . Increased level of HCV-RNA during chemotherapy were observed in all 12 pts and median level after reactivation was 4. $6x10^6$ copy/ml. Increased level of transaminases(ALT, AST) during chemotherapy were observed in all 12 pts and was from 3 to 50 time. When increased level ALT all 12pts were treated by antiviral therapy α - interferon 3ME once and ribaverin 800 mg daily. Decreased level of ALT and HCV-RNA was reached in all pts. In 2 pts IHC «+» was achieved molecular complete remission B-CLL and hepatitis C infection. In 10 patients with B-CLL IHC «-» was achieved only stabilization B-CLL. **Conclusions.** IHC analysis of B-CLL in patients with HCV infection is a determining factor for defining antiviral therapy as ferste line therapy as key method of treatment.

1305

EFFICACY OF RECOMBINANT HUMAN ERYTHROPOIETIN IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH ANEMIA

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Background. Anemia in Chronic Lymphocytic Leukemia (CLL) is a frequent symptom and can influence the efficacy of chemotherapy, survival rate and overall quality of life (QoL). Red blood cell (RBC) transfusions are routinely used to treat anemia, while Recombinant Human Erythropoietin (rHuEPO) treatment has been shown to significantly increase hemoglobin (Hb) concentration, reduce the number of RBC transfusions and improve QoL in patients with chemotherapy induced anemia. **Aims.** To study the efficacy of rHuEPO in CLL patients with anemia: increasing Hb concentration and anemic symptoms. **Methods.** In this study were included 26 CLL patients (stage C by J. Binet) had been diagnosed between 2005-2011. The median age of patients was 66 years (48-80). All patients had anemia with initial Hb concentration <10. 0 g/dL. The RBC transfusion threshold were Hb <8. 0 g/dL. rHuEPO (erythpoietin beta) was administrated subcutaneously in dosage 30. 000 IU one times a week. The effectiveness of rHuEPO-therapy was estimated by Hb concentration, red blood cells count and hematocrit (Ht) concentration and so anemic symptoms. The target Hb level was 12. 0 g/dl and planned duration of rHuEPO-therapy within 16 weeks. Positive response was considered as 1) transfusion-independency in patients earlier needed RBC transfusions, 2) increase Hb concentration <2. 0 g/dL. All patients earlier received 3 cycles of chemotherapy or more. Patients who had deficiency of iron, vitamin B_{12}, severe hemolysis (bilirubin >30 μmol/L), uncontrolled arterial hypertension were excluded. **Results.** Mean baseline Hb concentration was 8. 24±1. 65 g/dL (4. 6-10. 0 g/dL), RBC count - 2. 78±0. $64×10^{12}$/L (1. 36-3. $40×10^{12}$/L) and Ht - 24. 6±7. 4% (15. 4-32. 6%). In whole group of CLL patients (n=26) positive response was observed in 15 patients (57. 7%) and their Hb concentration, RBC count and Ht significantly (p<0. 05) increased from baseline to 12. 25±1. 17 g/dL (10. 7-14. 1 g/dL), 4. 07±0. $60×10^{12}$/L (3. 11-4. $90×10^{12}$/L) and 37. 6±3. 4% (32. 3-43. 4%), respectively. 12 patients had severe anemia (Hb <8. 0 g/dL) therefore they received 1-12 RBC transfusions during latest 1-3 months. During the study period (within 16 weeks) out of 13 patients 8 (61. 5%) were keeping transfusion-dependency, Hb concentration of these patients was depended on RBC transfusions (7. 9-10. 0 g/dL). However 5 (38. 5%) anemic patients significant increased Hb and stop doing RBC transfusions. Besides most patients with positive response reduced such symptoms as feeling fatigue (from 73. 1% to 38. 5%), weakness all over (from 50. 0% to 38. 9%), having trouble starting things because of tiredness (from 46. 2% to 26. 9%), drowsiness (from 73. 1% to 26. 9%), giddiness (from 46. 2% to 23. 1%), headaches (from 50. 0% to 23. 1%), pain in thorax (from 65. 4% to 34. 6%). Two patients with positive response in 6 weeks needed in repeated of rHuEPO administration because of anemia relapsed (Hb concentration decreased to 10. 0 g/dL). Nevertheless this way of persistent treatment could prevent RBC transfusions. No one case of thrombosis were observed during the period above 6 months. But four patients (26. 7%) with positive response had arterial hypertension (increasing more 20 mm/Hg) and needed in administration of hypotensive remedies. **Summary.** rHuEPO-therapy is effective treatment to reduce RBC transfusion, increase Hb concentration and decrease anemic symptoms in CLL patients with anemia.

1306

THE CLINICAL FEATURES OF 105 CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS FOLLOWED UP AT A SINGLE CENTER IN TURKEY

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Background. Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in western countries. CLL is a disease characterized by the clonal proliferation and accumulation of neoplastic B cells in peripheral blood, bone marrow, lymph nodes and the spleen. **Aims.** We evaluated the clinical features, survival and factors affecting prognosis in CLL patients followed up at our center. **Methods.** We included 105 CLL patients diagnosed at Trakya University Medical Faculty, Division of Hematology, Edirne, Turkey. The diagnosis of CLL was based upon the criteria of International Workshop on CLL. The demographic data, clinical features, treatment modalities and response to treatment were recorded from hospital files. Rai staging system was used for clinical staging. The definitions of partial (PR) and complete response (CR) were the criteria of National Cancer Institute Working Group. **Results.** Of 105 CLL patients, 65 were males and 40 were females (M/F=1. 62). The median age of the patients was 64. 8±9. 1 years (range:37-88). According to Rai staging system, 22 patients (21%) were stage 0, 18 (17. 1%) were stage I, 19 (18. 1%) were stage

II, 25 (23. 8%) were stage III, and 21 (20%) were stage IV. Autoimmune haemolytic anemia was diagnosed in 8 (7. 6%) CLL patients. Six patients developed a secondary malignancy, and 4 had Richter's transformation. Eighteen (30. 5%) of early stage Rai patients and 46 (100%) of advanced stage patients were decided to be given chemotherapy. The initial treatment modality in 23 cases was chlorambucil, it was fludarabine plus cyclophosphamide in 15 cases, COP in 12 cases, CHOP in 9 cases, and fludarabine only in 5 cases. Of early stage patients at initial diagnosis, 6 (33. 3%) had CR and 5 (27. 8%) had PR to initial treatment modalities. Four (8. 9%) of advanced stage patients had CR and 17 (37. 8%) had PR to initial treatment modalities. Unresponsiveness to initial treatment modalities was significantly more frequent in advanced stage patients (24 cases, 53. 3%) than in early stage patients (6 cases, 33%) ($p<0.05$). The duration of median follow-up was 40 months (1-140 months). The median duration of overall survival according to disease stages at the time of initial diagnosis was 112 months in early stage disease (Rai 0,I,II) and 68 months in advanced stage disease (Rai III,IV). The 5-year survival in early stage CLL was 86. 5% and in advanced stage patients it was 54%. Patients younger than 60 years at the time of diagnosis had significantly longer survival than elder patients (120 and 76 months, $p=0.01$). Patients with initially high LDH levels tended to have shorter survival than others (106 and 42 months, $p=0.001$). According to Cox multivariable regression analysis, being older than 60 years at the time of initial diagnosis (OR:3. 2, $p=0.014$), high LDH level (OR:6. 25, $p=0.01$) and advanced stage Rai disease (OR:5. 9, $p=0.01$) were independent poor prognostic parameters. **Summary and Conclusions.** The analysis of 105 CLL patients followed up at our center revealed that older age and advanced stage at the time of initial diagnosis were independent poor prognostic parameters.

1307

FIRST LINE TREATMENT IN CLL PATIENTS. PROSPECTIVE STUDY ABOUT DECISION CRITERIA AND CLINICAL PRACTICE IN GALICIA

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Introduction. Population in Galicia is estimated in 2. 795. 422 habitants with a median age of 45. 41 years. The prevalence of CLL is stimated in 2,2/100. 000 hab. (data of previous study). Data from were collected from 4 general hospitals during september 2009 to march 2011. **Patients.** 21. Median age: 66. 9 years old (46,0 - 86,0). Sex: Male: 66. 7 % - Female: 33. 3 %. All were Caucasian. None of them presented familial history of Non Hodgking Lymphoma no CLL. Criteria: B symptoms: 77% (fatigue: 33. 3 %; weight loss: 23. 8 %; night swears: 9. 5 %); Binet stage; Lymphocyte doubling time (100%); Lymphadenopathy: 84,6%. Splenomegaly: 47,6% Bulky disease: 16,7%; ECOG (100%); Bone marrow pattern: 61,1% (Diffuse: 18,2%; Interstitial: 36,4%; Nodular,diffuse: 18,2%); FISH: 61,1% (17 p:2 pts; 11q: 3 pts; 13q: 5 pts; +12: 5 pts. IGHV mutation status: not determined. Treatment: Median time from diagnosis to treatment was 19,4 months (0. 0- 90,1). FCR: 42,9 % (9 pts). Median time from diagnosis to treatment: 2,1 meses (0,0- 4,2). Median age: 60 years old (46,0- 72,0). Median lymphocyte doubling time: 3 months. Binet B: 44,4% . ECOG 0 (55,6%). No Bulky disease. Pattern Diffuse (40%). FISH: 13q (50%); +12 (50%). Estimated cycles: 5-6. Treatment completed in 100% of patients at full dose. FC: 4,8% (1 pt.). Characteristics were similar to patients included in FCR. Completed 6 cycles at a dose 100%. Chemotherapy with/without anthraciclins: 28,6% (6 pts). Median time from diagnosis to treatment: 30,5 months (12,2- 52,2). Median age: 70,3 years old (54,0- 82,0). Median lymphocyte doubling time: 23,2 months (4,0- 48,0). Binet A: 66,7%. ECOG 1 (50%). Generalized lymphadenopathy. Pattern Interstitial (100%). Estimated cycles: 5-6 Dose: 100% (3 pt); 75% (1pt); No data (2 pts). Completed in 90 % of patients. Alemtuzumab: 9,5% (2 pts). FISF: 17p. Treatment was not completed (adverse effects). The other schemes: Chlorambucil (1 pt); Chlorambucil + Prednisone (1 pt); Cyclofosfamide + Rituximab + Dexametasone (low dose) (1 pt) were considered in older patients with Unfit condition. **Conclusions.** Most criteria used B Symptoms; Binet stage; Median lymphocyte doubling time; Lymphadenopathy/splenomegaly; pattern of bone marrow infiltration and FISH analysis. Most frecuent scheme was FCR (included younger patients with rapid disease progression; Binet B and C; a diffuse pattern in B. M. and shorter lymphocyte doubling time). Median duration of treatment: 5. 1 months (median number of cycles 5,6). Chemotherapy was administer to older patients with longer lymphocyte doubling time; Binet A; generalized lymphadenopathy and an interstitial pattern of infiltration in B. M. Patients with 17p (2) were considered candidates to Alemtuzumab. Data showed that 85% of patients have completed the treatment with a Complete Response (CR- NCI WG criteria) of 52,4% (11 pts.) and Partial Response (PR) in 28,6% (6 pts.). Best responses were obtained in patients who finished all the programmed cycles at a dose of 100%. None of the patients underwent Bone Marrow Trasplantation nor Radiotherapy.

1308

LONG MAINTENANCE THERAPY OF INTERFERON IN HAIRY CELL LEUKEMIA

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Historically, the first treatment choices for hairy cell leukemia (HCL) were splenectomy and α -interferon. In this study we evaluate the efficacy of long-term maintenance therapy of α -interferon (IFN) in hairy cell leukaemia (HCL), a disease that remains incurable. **Methods.** 8 patients (5 male, 3 female, aged 45-62 yrs) with hairy cell leukaemia (HCL) from 1994 to 1996 beginning the therapy of IFN 3mU 3 times weekly. Complete remission has been reached at all pts. Therapy has been then continued as long-term maintenance therapy INF once time 3 mU weekly. **Results.** All 8 patients are in a state of complete response at 14-17 years (median 15 years). No incidence of second cancers of this 8 HCL pts has not been documented. **Conclusions.** We hope that these reports will lead to a multi-centre, phase III study of IFN maintenance therapy (including pegylated IFN, given less frequently) in HCL patients achieving optimal response to initial therapy, be it IFN or a purine analogue.

1309

CELL SURFACE PHENOTYPE OF CD34+ PROGENITORS IN CHRONIC MYELOID LEUKAEMIA: POTENTIAL FOR DIAGNOSIS AND FOLLOW-UP

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Background. Chronic myeloid leukaemia (CML) is characterized by the translocation t(9;22)(q32;q34) resulting in the BCR/ABL fusion gene. The diagnosis of CML is based on the detection of this translocation either by cytogenetic or molecular techniques. Only a few flow cytometric studies have examined the immunophenotype of CML progenitors, but some interesting aberrancies were seen. **Aims.** To assess the diagnostic potential of immunophenotyping $CD34^+$ cells in CML, considering this could be a useful tool to reduce the number of cytogenetic/molecular tests needed. In addition, we studied the changes in the phenotype that occurred during treatment of patients. **Methods.** All bone marrow (BM) aspirates, taken in our hospital between 2000 and 2009 for suspicion of a chronic myeloproliferative neoplasm and for which also an analysis for the presence of BCR/ABL was performed, have been included in this study. Samples from CML patients at diagnosis (n=17), BCR/ABL negative myeloproliferative neoplasms (n=42), myelodysplastic/myeloproliferative neoplasms (n=5) and from patients with non-neoplastic myeloproliferation (n=8) were examined. The results were compared with samples obtained in a control population, consisting of healthy BM donors and cardiosurgery patients (n=32). In addition, BM samples from CML patients treated with hydroxyurea (n=7) or imatinib (6 patients, 16 samples) were studied. $CD34^+$ cells were enumerated and their expression of cell surface antigens was determined with flow cytometry. The following antigens were analyzed: the B-cell lineage markers CD19 and CD10, the myeloid markers CD13 and CD33, the stem cell marker CD133, the stem cell factor receptor (CD117), the interleukin-3 receptor alpha (CD123), the chemokine receptor CXCR4 (CD184) and the common leukocyte antigen (CD45). Results were expressed as relative mean fluorescence intensity (RMFI). Reference values were defined for each parameter as the range of results obtained in the control population. **Results.** At diagnosis, the $CD34^+$ cells in all CML patients showed a CD133 RMFI below that of the normal population. This low CD133 expression identified CML patients within all patients with myeloproliferation with 100% sensitivity and 87. 3% specificity. Only 7 out of 55 patients with BCR/ABL negative myeloproliferation showed the above abnormality. Therefore, cytogenetic/molecular tests for t(9;22) were only needed to differentiate the latter patients from CML in the 'CD133 low' group (33% of all samples). In addition $CD34^+$ cells of CML patients at diagnosis showed high levels of CD33 and CD45, and a low positivity for CD19, CD10 and CD123. Treatment with hydroxyurea induced no changes in the phenotype. In CML patients treated with imatinib CD10, CD33 and CD45 levels normalized. CD133 levels increased compared to diagnosis but remained lower than normal. CD19 and CD117 RMFI were respectively higher and lower compared to normal controls. **Conclusions.** CML progenitor cells display distinct immunophenotypic abnormalities, predominantly characterized by low CD133 expression. These phenotypic characteristics could help to reduce the number of patient samples for which molecular techniques are required. With

a more extensive panel of antibodies, it might be possible to identify CML with higher specificity. The diagnostic immunophenotype of CML largely normalizes under imatinib treatment.

1310

ZOLEDRONATE INDUCES APOPTOSIS VIA EFFECTING ON STAT PATHWAY IN CHRONIC MYELOID LEUKEMIA CELLS

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Background. Although development of tyrosine kinase inhibitors has revolutionized the CML therapy, resistance to targeted drugs is a frequent clinical problem. To overcome this, targeting alternative downstream regulators of BCR-ABL is probably the best therapeutic strategy. **Aims.** In this study, we aimed to evaluate the cytotoxic and apoptotic effects of Zoledronate on K562 human chronic myeloid leukemia cells and to examine the roles of signal transducers and activator of transcription proteins (STATs) on Zoledronate-induced apoptosis. **Methods.** Cell viability and cytotoxicity tests were conducted by using Trypan blue dye exclusion and XTT assays, respectively. Apoptotic analyses were performed by using AnnexinV-EGFP staining method under fluorescence microscopy. Expression levels of STAT3, -5A and -5B genes were analysed in Zoledronate-treated K562 cells by qRT-PCR. To confirm the data, protein expressions of mentioned genes were evaluated by using Western Blotting. **Results.** The results showed that Zoledronate decreased viability and proliferation and induced apoptosis in K562 cells in a dose- and time-dependent manner as compared to untreated controls (Figure 1). The IC50 value of Zoledronate is found to be as 60 microM. Concurrently, the expressions of STAT3, STAT5A and STAT5B genes both in mRNA and protein levels have significantly been reduced in Zoledronate-treated K562 cells as compared to untreated controls. **Summary and Conclusions.** These data indicated that STAT inhibition by zoledronate is the underlying mechanism of Zoledronate-induced apoptosis of K562 cells. More importantly, we have shown for the first time that zoledronate triggers apoptosis through inhibiting the expression levels of STATs in a hematological malignancy. If it can be conveyed to clinical area, zoledronate may contribute to the treatment of CML as a promising therapeutic agent.

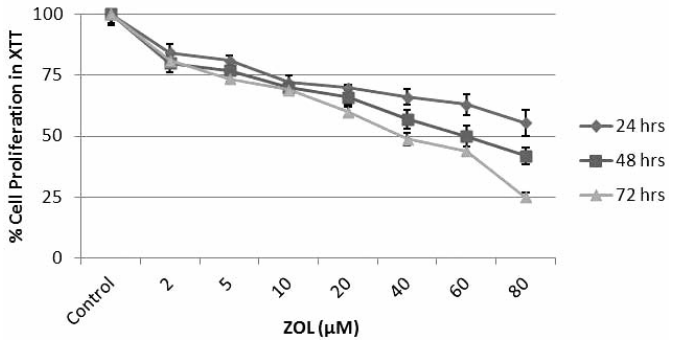

Figure 1. The cytotoxic effect of Zoledronate in K562 cells in dose and time dependent manner.

1311

MUTATIONAL ANALYSIS OF THE FOCAL ADHESION ADAPTOR PROTEIN PAXILLIN IN CML

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Background. Pathogenesis of chronic myelogenous leukemia may not only involve increased proliferation of stem cells/myeloid progenitors but at the same time impaired homing/adhesion of myeloid progenitors to the bone marrow stroma. Altered focal adhesion to stroma could be associated not only with premature release to the periphery, but also with altered proliferation and/or differentiation procedures, since cell-matrix adhesions are specific types of macromolecular assemblies through which both mechanical force and regulatory signals are transmitted. One of various key molecules within focal adhesions is the adaptor protein paxillin, whose tyrosine phosphorylation from FAK (focal adhesion kinase) and Src participates in the actin polymerization/depolymerization procedure. Several reports have linked BCR-ABL kinase activity to FAK and Src phosphorylation, while somatic paxillin mutations have been found in lung cancer with a frequency of 10% in a reported series, the most frequent of them (A127T) residing between its LD1 and LD2 motifs and being absent in normal tissues of the same patient series. Paxillin A127T mutation was shown to enhance tumor growth and invasion in vivo (engraftment of a H522 cancer cell line harboring the mutation or not in nude mice). **Methods.** DNA samples from 41 CML patients prior to therapy with BCR-ABL kinase inhibitors were subjected to mutational analysis of the most frequently mutated region of the paxillin gene (between LD1 and LD2 motif) using PCR amplification and direct sequencing. **Results.** In the 41 samples tested, the A127T mutation was found in one sample. No other mutations were detected within this region, while mutations at different positions within the same region have been reported in other cancers. The sample carrying the A127T mutation corresponded to a patient who developed diphenotypic acute leukemia six years after diagnosis, having received no TKI therapy, as she died shortly before their introduction into clinical practice. **Summary and Conclusions.** Mutation of the paxillin gene, at least at a frequently mutated position in lung cancer, is a rare event in CML. Its significance for paxillin expression as well as the role of paxillin expression in the response to therapy with TKI inhibitors remains to be determined in our ongoing study. This is the first report of the A127T mutation in a haematological malignancy and further sample sequencing and comparison with non-relevant samples is under way.

1312

CHRONIC MYELOID LEUKEMIA WITH VARIANT PHILADELPHIA TRANSLOCATION AND CONSTITUTIONAL PERICENTRIC INVERSION BOTH OF HOMOLOGOUE CHROMOSOMES 9

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Background. Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of the Philadelphia (Ph) chromosome resulting from the reciprocal translocation t(9;22)(q34;q11). In 5-10% of patients with newly diagnosed CML, additional chromosome(s) combine to generate the composite, so-called variant translocations. Some previous studies have suggested that patients with variant Ph translocations may have inferior prognosis to those with classical traslocations. Other have reported the lack of prognostic impact of variant Ph translocation in imatinib mesylate (IM)-treated patients in the chronic phase (CP). In contrast to the Ph chomosome, constitutional pericentric inversion of chromosome 9 [inv(9)(p11-12q13)] has been considered as a normal familiar variant in human genome. The incidence varies with ethnicity and is approximately 0,8 to 2%. A thorough study of the heterochromatin organization in the pericentric region has revealed a homology between 9p11-12 and 9q13-21. Such homologous sequences may participate in the mechanism generating the inversion. Whether constitutional inv(9) predisposes to cancer remains controversial. Because inv(9) is not considered an important finding, a bias in reporting it may account for the paucity of references to on constitutional inv(9) in hematological disorders. **Aims.** Report of association of the variant Ph translocation and the constitutional bichromosomal inv(9) in a newly diagnosed CP CML patient. **Methods.** A 54-year-old female was diagnosed with CP-CML in October, 2008. Cytogenetic and FISH studies were done on the bone marrow cells prepared according to standard techniques and using DNA probes that hybridize at the BCR and ABL regions. Constitutional karyotype was confirmed in phytohemagglutinin-stimulated peripheral blood lymphocytes. Karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN 2009). Isolated RNA sample was transcribed into cDNA and tested for p210 transcript by Real Time PCR. **Results.** The diagnostic karyotype 46,XX,t(5;inv(9)c;22)(q13;q34;q11), inv(9)(p11q13)c was found in 20/20 analyzed metaphases. The banding confirmed that variant Ph chromosome resulted from a complex translocation including chromosomes 5q13, inv(9) and 22q11. Another homologue 9 also was inverted. The constitutional karyotype obtained from the peripheral blood lymphocytes was 46,XX,inv(9)(p11q13)c,inv(9)(p11q13)c[22]. This novel variant translocation incorporating chromosomes 5q13, inv(9), and 22q11 was confirmed by FISH. The patient was treated with IM as a first-line therapy (400 mg/d) and after 6 month obtained a complete cytogenetic response. She still remains in the CP-CML. **Conclusions.** In conclusion, diploid form of constitutional inv(9) is a truly random chromosomal aberration. As far as we know, the karyotype of our patient is unique because it contains inverted chromosome 9 on both homologues, and one of them is affected by a variant Ph translocation. Our patient treated with IM as a frontline therapy confirm that the clinical characteristics and outcome of patients with variant Ph

translocations are similar to those with classic Ph translocation. Therefore, our results suggest that patients with variant translocation do not constitute a 'warning' category in the imatinib era.

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STUDY OF THE RELATION BETWEEN CD34 AND CD7 EXPRESSION ON MARROW STEM CELLS OF CML PATIENTS AND THE BCR-ABL TRANSCRIPTS COPY NUMBER

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Background. Among CML patients who achieve BCR-ABL transcript-undetectable status; few remain transcript negative after molecular target therapy is stopped while others show re-appearance of transcripts. Quiescent leukemic stem cells are responsible for recurrence. This suggests the origin of malignant leukemic cells may be variable. **Aims.** To study the relation between the expression of early myeloid progenitor marker; CD7 on CD34 CML cells, the prognostic Sokal score and BCR-ABL transcripts level aiming to find out if the origin of stem cells is related to any prognostic factor or the BCR-ABL load. **Patients and Methods.** This study was conducted on a group of 34 Egyptian patients newly diagnosed to have Chronic myeloid leukemia in chronic phase from clinical hematology unit of Kasr El Einy teaching hospital. They did not receive TKIs or interferon prior to sampling. RT-PCR for BCR-ABL and Dual culture Epics XL Flucytometry; using dual color, FITC for CD34 and PE for CD7 on bone marrow aspirate samples. **Results.** Thirty one of 34 patients (91%) have CD34 +ve with mean expression 10. 5918%. Twenty two patients (65%) have dual CD7 and CD34 expression with mean 1. 6256%. Dual CD7 and CD34 expression didn't correlate with Sokal score or BCR-ABL transcripts copy number. CD34 expression correlated significantly with transcript copy number (P:0. 04). **Conclusions.** The CD34 expression in CML may bear a relation to the initial BCR-ABL transcripts number. This may impact the preponderance of recurrence of disease on stoppage of tyrosine kinase inhibitors treatment.

1314

MDR1 GENE EXPRESSION MONITORING BY RQ-PCR IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA RESISTANT TO IMATINIB THERAPY

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Background. Chronic myeloid leukemia (CML) is a clonal disorder of the hematopoietic stem cells, characterized by the presence of *BCR-ABL* fusion gene. Imatinib mesylate is now in widespread use for the initial treatment of chronic phase of CML. In most patients this therapy allows for achievement of cytogenetic, and deep molecular response, however, this is not possible in remaining patients. Several molecular mechanisms of resistance to imatinib has been described. The most important are mutations occurred within *ABL* kinase domain of *BCR-ABL* gene, the other could be low *hOCT1* and high *MDR1* genes expression responsible for low effective intracellular imatinib concentration . *MDR1* (multidrug resistance gene 1) gene is one of the several members of ABC transmembrane transporter family ABCB1. *MDR1* gene encodes P-glycoproteine (Pgp) which is responsible for imatinib efflux from the cell. **Aims.** The aim of the study was to develop the RQ-PCR-based method allowing for *MDR1* expression measurement using the TaqMan Technology. **Methods.** 11 CP-CML (chronic phase CML) patients resistant to imatinib were included in the study. White blood cells were collected by using lyses erythrocytes method. Total RNA was extracted using TRIzol method and then reverse transcription was performed (with use of SUPERSCRIPT II, Life technologies). *MDR1* gene expression was detected by Real Time PCR. RQ-PCR was performed using TaqMan Master Mix (Life technologies) and primers and probe as described by Galimberti S et al (MDR1 forward primer 5`-TGC AGC ATT GCT GAG AAC ATT, MDR1 reverse primer 5` TGC CCT CAC AAT CTC TTC CTG, MDR1 probe 6FAM-CCT ATG GAG ACA ACA GCC GGG TGG TT-TAMRA) on ViiA 7 Dx platform. *MDR1* gene expression levels were normalized with *ABL* gene (primers and probes as described in *EAC* protocol, Gabert et al.) expression levels in the same sample. CEM VBL 100 cell line (CEM vinblastine treated cell line was kindly provided by Dr Wiliam T. Beck) was adopted as a reference. 2^-ddCt method was used to calculate the results. *MDR1* expression level of CEM VBL was arbitrally defined as 100 AU (arbitrary units). *MDR1* expression in CML patients` results obtained in the study were compared. by relating them to CEM VBL cell line. **Results.** All CP-CML patients resistant to imatinib showed low level of normalized *MDR1* expression. The range was 1,23 - 11,03 AU, median was 3,67. The parallel analysis of mutational *ABL KD* gene status in this group of patients did not show the presence of any mutation. However, in 2 patients high level of *hOCT1* expression was observed. **Conclusions.** RQ-PCR based methodology developed within this study is feasible and allows for the *MDR1* gene expression analysis. The molecular mechanisms of resistance to imatinib in studied group of patients was not related to the alteration of *MDR1* gene expression. In 2 patients low level of *hOCT 1* expression is the most possible cause of imatinib resistance. In remaining 9 patients the resistance mechanisms were not recognized.

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EFFICACY OF NILOTINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE WITH AND WITHOUT PH1 VARIANT TRANSLOCATION

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Background. Chronic myeloid leukemia (CML) is characterized by Philadelphia (Ph) chromosome with a chimeric gene BCR-ABL. Variant Ph chromosome translocations involving chromosomes other than 9 and 22 are found in 5-10% of CML cases. Nilotinib is a tyrosine kinase inhibitor with high target specificity approved for use in patients with newly diagnosed CML in chronic phase (CML-CP). **Aims.** In this study we evaluated the efficacy of Nilotinib in Ph1 positive chronic myeloid leukemia patients with classical or complex translocation. **Methods.** We here report a group of 7 patients (4 males, 3 females) with a median age of 44 that receive Nilotinib during a median follow up period of 12 months (range 6-21 months). The evolution of patients with CML-CP was evaluated to determine the efficacy and tolerability of Nilotinib. **Results.** Five patients (72,4%) presented classical Ph1 created by reciprocal t(9;22)(q34;q11) translocation and 2 patients (28,6%) have a complexe Ph1 translocation. One of the cases has mosaic translocation with 2 clones t(8;9;19;22) and t(9;19;22) and the other case has omogen translocation t(7;9;15;22). All patients achieved a hematologic response after 4 months of Nilotinib treatment. Complete cytogenetic response (CCyR) was achieved in 100% of the 7 patients, while for cases with classical Ph1 translocation after 4 months and for cases with variant Ph chromosome translocation after 10 months. Responses were durable, with 100% of patients maintaining CCyR in courses of follow-up. **Conclusions.** We found out that nilotinib was efficacious in chronic phase CML patients and it was also successful in CML patients with a variant of Ph chromosome translocations. These findings indicate CCyR rates higher for nilotinib. Administration of nilotinib offered an effective treatment in a CML patients with a variant of Ph chromosome translocations.

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P190 BCR-ABL IS ASSOCIATED WITH INFERIOR OUTCOME IN CHRONIC MYELOID LEUKEMIA

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The most common bcr-abl transcript in chronic myeloid leukemia(CML) are e13a2 and e14a2 which are transcribed in P210 protein. e1a2 transcript is reported in acute lymphoblastic leukemia (ALL) and rarely in CML. We analyzed 470 CML Tunisian patients for bcr-abl transcript isoform. Results showed that 45% of patients express the isoform e13a2, 51% express the isoform e14a2, and identified only 1%(3 patients) with e1a2 isoform patients . These 3 patients were in accelerated phase and never reached the major molecular response with tyrosine kinase inhibitors. These results confirm the data from the literature which agree on the pejorative prognosis of CML expressing the P190 isoform. CML with P190 rearrangement is rare and is associated with an inferior outcome. These patients need to be identified as high risk patients. In conclusion the identification of variant bcr-abl is a crucial step in the diagnosis of CML since it allows reliable monitoring of residual disease. Since the era of second generation ITK, patients with P190 should be considered as high-risk forms and benefit of increased therapeutic.

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INCIDENCE OF SECOND MALIGNANCIES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) IN CLINICAL PRACTICE ANALYSED IN THE FRAME OF INTERNATIONAL RESEARCH PROJECT EUTOS IN RUSSIAN FEDERATION (RUS)

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Background. Success of tyrosine kinase inhibitors (TKIs) in CML has given patients(pts) hope for a long disease free survival. With the increase in survival can be revealed, such long-term effects of TKI treatment as the development of other tumors. Some pts have a history of previous cancers by the time they are diagnosed with CML. According to the literature, the incidence of secondary tumors varied from 1,02% (Kim DW, et al. Blood 2009) up to 4,02% (Verma D, et al Blood2008); similar in terms of a cohort of pts analyzed by the research group GIMEMA (Gugliotta G, et al. Blood 2010). **Aims.** To evaluatethe incidence and variants of second malignancies(SM) in CML patients treatedwith imatinib(IM) frontline in clinical practice in RUS. **Methods.** Analyzed data from 29 administrative districts of RUS. The selection of regions based on the quality of the data from CML pts registry. 608 CML pts were included in this study with the criteria (EUTOS OSP-study): Ph'/bcr-abl-positive CML diagnosed in 2002-2006, age of pts ≥ 18 years (y), initiation of IM therapy ≤6 months (mo) from the date of diagnosis. Median (Me) age was 48(18- 82) y, sex ratio (M/F(%)) 288/320 (47/53) pts, Me time from diagnosis to IM therapy was 2. 4(0- 6) mo. Pretreatment: Hydrea 461 (76%) pts; Mielosan 4 (0. 6%) pts, chemotherapy 21(3. 5%) pts, IFN-α 37 (6%) pts. Me follow-up since the beginning of CML treatment was 55. 2 (1- 108) mo (*6 pts have not data on the date of analysis). Statistical analysis performed using a package SAS9. 1. 3. **Results.** The cumulative incidence of SM in our CML pts was 2. 9% (17 pts). It should be noted that the identified cases were divided into subgroups: tumors detected before the diagnosis CML (prior malignancies, (pM)) and after (SM). Three out of observed incidences- pM (lung cancer, thymoma, stomach tumor) were revealed and treated surgically in 11, 10 and 19y respectively, before diagnosis of CML; 2 pts are alive (observation time since diagnosis of CML- 83 and 78 mo), treated by IM as a first line with complete molecular response (CMR). In 14 patients were identified SM; sex ratio (M/F) 8/6pts. Me age at the diagnosis of CML was 58(extr. 44- 75)y; Me time detection of SM since diagnosis of CML was 31 (extr. 0- 57) mo. Me time of IM therapy 58 (14- 71) mo. The most common SM: breast cancer (4 of 17 cases) and tumors of the gastrointestinal tract (4 of 17 cases). Most of the pts (among 17 incidences) had good results of TKI therapy: 11 pts achieved major and complete molecular response (Me 37 (extr. 6- 67)mo). Six out of the 608 (1%) pts or 35% of 17 pts died due to second neoplasmprogression in stable CP CML (1pt- pM/5pts- SM). **Conclusions.** Identified cases of a combination of malignancies and CML are of interest. The study of this aspect of CML will allow a deeper understanding the relationship between the development of SM, leukemia and their therapy.

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TYROSINE KINASE INHIBITORS FOR THE TREATMENT OF CHRONIC MYELOID LEUKAEMIA: A SYSTEMATIC REVIEW

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Background. Chronic myeloid leukaemia (CML) is a myeloproliferative disorder of blood stem cells. The tyrosine kinase inhibitor (TKI) imatinib was the first targeted therapy licensed for patients with chronic phase (CP) CML and it's introduction was associated with substantial improvements in response and survival compared with previous therapies. Clinical trial data are now available for the second-generation TKIs (nilotinib, dasatinib, and bosutinib) in the first-, second-, and third-line settings. **Aims.** To systematically review and qualitatively compare the clinical effectiveness, safety and effect on quality of life (QoL) of TKIs for the management of chronic, accelerated or blast phase CML patients. **Methods.** Included studies were identified through a search of electronic databases (accessed March 2011), and relevant conference proceedings. Outcome data were extracted by two independent reviewers. Primary outcomes of interest included complete cytogenetic response (CCyR), major molecular response (MMR), complete molecular response (CMR), transformation rates, incidence of adverse events (AEs) and QoL. **Results.** Of 3,248 potentially relevant citations, 47 publications of 23 unique studies met the pre-defined inclusion criteria (11 randomised controlled trials (RCTs) and 12 single-arm studies). All RCTs enrolled CP CML patients: eight investigated first-line treatments (imatinib, n=4; dasatinib, n=2; nilotinib, n=1; bosutinib, n=1) and three second-line treatment with dasatinib. In the first-line setting, the long-term efficacy (up to eight years) of imatinib has been confirmed in a single RCT (IRIS). In contrast, 2-year follow-up data on the second-generation TKIs have recently been published from three imatinib-controlled Phase III RCTs (nilotinib [ENESTnd study, n=846 enrolled patients]; dasatinib [DASISION, n=519]; bosutinib [BELA, n=502]). All second-generation TKIs reported lower rates of transformation, and comparable or superior cumulative CCyR, MMR and CMR rates compared with imatinib by 2-years follow up. Each of the second-generation TKIs was associated with a distinct AE profile compared with imatinib: nilotinib with increased incidence of rash, dasatinib with certain haematological AEs and bosutinib-treated patients reporting increased gastrointestinal AEs. Bosutinib was the only second generation TKI to report QoL data (no statistically significant difference compared with imatinib). Of the 12 single-arm studies, eight investigated second-line treatments (dasatinib, six studies, n= 916 enrolled patients; nilotinib, n=280; bosutinib, n=288), three third-line (nilotinib, n=60; bosutinib, n=170; nilotinib/dasatinib, n=26) and one study enrolled both second- and third-line patients (nilotinib/dasatinib, n=48). Data in the second- and third-line setting confirmed the efficacy of the second-generation TKIs in either imatinib resistant or intolerant patients as measured by CCyR and MMR rates. **Summary and Conclusions.** Data from first-line RCTs reporting up to 2-year follow up indicate superior response rates of the second-generation TKIs compared with imatinib. However, whether this superior efficacy over imatinib is maintained over longer treatment periods and is associated with improved overall survival remains to be confirmed. There is currently a paucity of QoL data in the first-line setting, with bosutinib the only second-generation TKI to have published RCT evidence. Current evidence from single arm studies in the second-line setting confirm that nilotinib, dasatinib, and bosutinib are valuable treatment options for the significant subgroup of patients who are intolerant or resistant to imatinib treatment.

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THE DIFFERENCES AND CORRELATIONS OF BCR-ABL TRANSCRIPTS BETWEEN PERIPHERAL BLOOD AND BONE MARROW ASSAYS ARE ASSOCIATED WITH THE MOLECULAR RESPONSES IN THE BONE MARROW FOR CHRONIC MYELOGENOUS LEUKEMIA

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Background. Previous studies concerning *BCR-ABL* mRNA monitoring by quantitative real-time RT-PCR (Q-PCR) for chronic myelogenous leukemia (CML) have shown a significant concordance between peripheral blood (PB) and bone marrow (BM) assays, confirming that it was not necessary to obtain BM aspirates for accurate molecular monitoring. **Aims.** To determine whether molecular monitoring by Q-PCR using PB is comparable to using BM during the treatment of CML. **Methods.** Between May 2006 and July 2011, a prospective comparative study was performed to analyze the Q-PCR results (*ABL* as a control gene) of 712 simultaneous paired PB and BM samples from 330 CML patients in the chronic phase or the accelerated phase before and during imatinib therapy. All patients provided written informed consent. The study protocol was registered in the Chinese Clinical Trial Registry (Registration Number: ChiCTR-ONC-11001697). **Results.** For the 78 paired pretreatment samples from 78 newly diagnosed CML patients, the level of *BCR-ABL* mRNA in the PB was lower than that in the BM (P=0. 007). Among the 634 paired on-treatment samples which were collected from 262 patients (10 patients were diagnosed and followed from before the treatment and to during the treatment) at a median follow-up of 18 months (range 3-120 months) from the initiation of imatinib therapy, although the overall amounts of *BCR-ABL* mRNA in the PB and BM samples were comparable (P=0. 072) and there was a strong correlation (r=0. 839, P<0. 001), the level of *BCR-ABL* mRNA in the PB was lower than that in the BM (P<0. 001) where BM *BCR-ABL* mRNA < 1 log-reduction from the baseline (n=78 pairs), comparable with that in the BM (P=0. 499) where BM *BCR-ABL* mRNA ≥1-<2 log-reductions (n=99 pairs), and higher than that in the BM (P<0. 001) where the BM *BCR-ABL* mRNA ≥2 log-reductions (n=457 pairs). A strong correlation (r=0. 811, P<0. 001) was only found where BM *BCR-ABL* mRNA <1 log-reduction. Overall, the depth of the molecular response (the log-reduction values for *BCR-ABL* mRNA: 2. 39 vs. 2. 71, P<0. 001) and the proportion of major molecular response (29. 3% vs. 38. 3% P=0. 001) in the PB were lower than those in the BM. A further analysis on the dynamics of the leukemia burden for the 55 patients revealed that the log-reduction values for *BCR-ABL* mRNA in the PB were com-

parable with those in the BM at 3 months (P=0. 975) and 6 months (P=0. 076), and lower than those in the BM after 12 months (all P values < 0. 05) of imatinib therapy. **Summary.** The differences and correlations of the levels of *BCR-ABL* mRNA between PB and BM assays were associated with the molecular response in the BM and Q-PCR monitoring using PB has a greater sensitivity than using BM, especially while monitoring the deeper molecular responses of CML patients during imatinib therapy. **Conflict of interest disclosure:** All authors declare no competing financial interests.

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MULTICENTER CLINICAL STUDY OF NILOTINIB IN CML PATIENTS WITH IMATINIB RESISTANCE OR INTOLERANCE: EVALUATION OF THE MOLECULAR RESPONSE BASED ON BCR-ABL MUTATION STATUS AND PLASMA TROUGH CONCENTRATION

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Background. The tyrosine kinase inhibitor (TKI) imatinib is used as first-line therapy for newly diagnosed chronic myeloid leukemia (CML). However, some patients fail to respond to imatinib or become intolerant to it. Nilotinib is a second-generation TKI with higher selectivity and more potent inhibitory effects on BCR-ABL than imatinib, and several studies have reported hematologic and cytogenetic responses with nilotinib in patients who cannot tolerate imatinib or whose CML is imatinib-resistant. **Aims.** The safety and efficacy of nilotinib at 400 mg bid was evaluated in imatinib-resistant and -intolerant patients with CML-chronic phase (CP) or -accelerated phase (AP) at The East Japan CML Study Group (EJCML). The response to nilotinib was evaluated based on the BCR-ABL mutation status and its plasma trough concentration. **Methods.** Nilotinib was administered orally at 400 mg bid for one year, and the molecular responses were monitored using the international standard method of quantitative PCR (IS-PCR). BCR-ABL mutation was analyzed by direct sequencing (DS) at baseline and after 12 months of nilotinib treatment or at the time of an event. Plasma trough concentrations of nilotinib were measured using HPLC after 3 months. **Results.** From March 2009 through February 2011, 51 patients were registered in the study, and CRFs were collected from 49 patients (33 imatinib-resistant and 16 intolerant; 46 CP and 3 AP) by February 2012. The median follow-up period was 11. 7 months (range: 0. 1-13. 3). Forty-nine patients were evaluated for a molecular response. The rates of MMR (≤0. 1% IS) were 30. 8% and 55. 0% at 6 and 12 months, respectively, and the CCyR equivalent (≤1. 0% IS) rates were 61. 5% and 85. 0% at 6 and 12 months, respectively. Using DS, 5 BCR-ABL mutations (M244V, F317L, N358D, F359V and E459K) were detected in 6 patients (12. 2%) at baseline, and the M244V, N358D, F359V and E459K mutations had disappeared after 12 months of nilotinib. On the other hand, acquired BCR-ABL mutations (Y253H, I418V and exon 8/9 35bp Insertion) were detected after 12 months in 3 patients (6. 1%) who did not achieve CCyR or MMR. No patients acquired a T315I mutation. In the safety evaluation, grade 3/4 hematological toxicity included anemia in 12. 2% of patients, neutropenia in 26. 5% and thrombocytopenia in 18. 4%. QTc prolongation at all grades was found in three patients (6. 4%). Thirty-nine (77. 6%) patients are still on treatment; reasons for discontinuation were progressive disease in one patient with F317L, insufficient effects in one patient with no mutations, and adverse events in 9 patients. Among those on treatment, the plasma trough concentration of nilotinib was significantly higher in patients with MMR than in those without it (mean: 1067. 4 ng/ml vs. 629. 8 ng/ml, *P*=0. 0328). And patients with MMR were significantly less in Q1 (<489. 7 ng/ml) than in Q2-4 (489. 7-2899. 9 ng/ml) (44. 4% vs. 80. 0%, *P*=0. 0447). **Summary.** Imatinib-resistant patients, even those with BCR-ABL M244V, N358D, F359V and E459K mutation, achieved MMR after nilotinib treatment. The plasma trough concentration of nilotinib was related to the response in this study. Nilotinib showed good efficacy and tolerability in Japanese imatinib-resistant and -intolerant CML-CP or -AP patients.

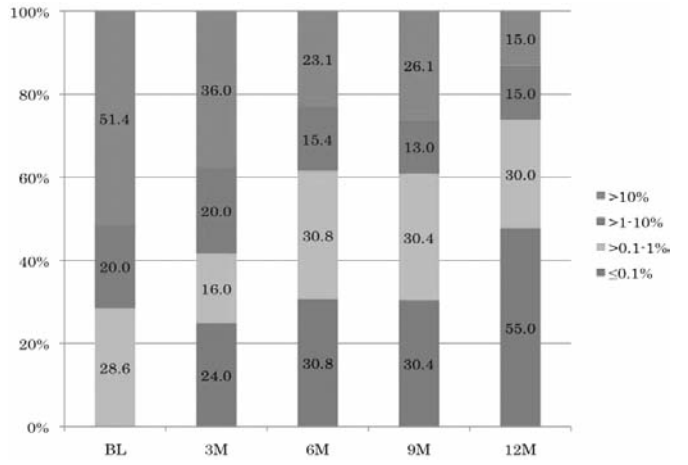

Figure 1.Complete Cytogenetic Response equivalent (≤1. 0% IS) and Major Molecular Response (≤0. 1% IS) to Nilotinib Over Time.

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HOMOHARRINGTONINE BLOCKS THE EPHB4/RAC1/CDC42/RHOA PATHWAY CONTRIBUTING TO IMATINIB RESISTANCE

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Background. At present, the homoharringtonine (HHT) + imatinib (IM) therapy may overcome disease-poor response to conventional doses of imatinib. However it is not still determined the mechanism of overcome IM resistance. In our pre-experiment stage, it was determined that a new marker of IM resistance mediated by the activation of EphB4/Rac1/cdc42/RhoA pathway. **Aims.** Our purpose is to address whether HHT contributes to block the EphB4-mediated pathway. **Methods.** The apoptosis rate of K562 IM-resistant (K562-R) cell was detected with PE Annexin. The IM inhibition rates of K562-R or K562-R+HHT were analyzed by MTT assay. BALB/C female nude were used in xenograft experiments. Phosphorylation of related protein was analyzed by Western Blot. **Results.** The apoptosis detection showed the apoptosis rate of K562-R was only 2. 35 ± 0. 11% co-cultured with IM (1. 2 mg/L), but the apoptosis rate reached 58. 71 ± 2. 39% with K562-R incubated with HHT□20ug/L□+IM (P<0. 001). MTT assay showed that K562-R was IM resistance (IC50 5. 45 mg/L), but HHT made K562-R cell become sensitive to IM (IC50 1. 17 mg/L, P<0. 001). K562-R xenograft volumes significantly decreased with IM+HHT treatment comparing with before treatment (1692. 82 ± 317. 14 mm^3 versus 975. 56 ± 132. 42 mm^3, P<0. 001). On the contrary, K562-R tumor volumes significantly increased with simple IM treatment comparing with before treatment (1733. 82 ± 399. 22 mm^3 versus 2301. 25 ± 555. 76 mm^3, P=0. 001). Overall survival after treatment termination was 0, 16. 7% and 83. 3% in the IM, HHT, and HHT+IM therapy groups, respectively (P<0. 001). The EphB4, phosphorylation of Rac1+Cdc42 and RhoA was significantly decreased after HHT incubate in K562-R cells (P<0. 001). Analysis of xenograft tissue demonstrated that the parallel results. The expressions of EphB4/Rac1/cdc42/RhoA began to decreased after HHT treatment (P<0. 001). 5. **Conclusions.** We demonstrated HHT enhanced the sen-

sitivity to IM for K562-R cell and xenograft. There were no differences in the phosphorylation of MEK/ERK using HHT culture. The EphB4 and phosphorylations of RhoA and Rac1+cdc42 were simultaneously decreased after HHT intervention in K562-R cell and xenograft tissue. HHT, as a conventional anti-leukemia drug, locked the EphB4/Rac1/cdc42/RhoA pathway.

1322

SECOND-GENERATION TYROSINE KINASE INHIBITORS AS SECOND LINE TREATMENT IMPROVE OUTCOME OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA RESISTANT OR INTOLERANT TO IMATINIB

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Background. Imatinib has offered an outstanding improvement in the prognosis of chronic myeloid leukemia (CML) patients. Nevertheless, the IRIS trial has shown that, at eight years, 22% are off-therapy because of resistance (16%) or toxicity (6%) (Deininger M, et al. ASH 2009;114:1126). Clinical trials have shown that second-generation tyrosine kinase inhibitors (2GTKIs) using as salvage therapy, can induce long-term progression-free survival (PFS) in almost 60% of the patients (Shah NP et al Haematologica. 2010; 95:232-240.). However, data on this topic are scarce outside of clinical trials. As second-generation tirosin kinase Inhibitors (2G TKIs) are being evaluated as first line treatment with quite favourable outcomes so long, it is important to know what has happened when 2G TKIs have been used sequentially for patients with previous unfavourable responses to Imatinib, outside the controlled clinical trial setting.

Index		Best CCtR		Best MMR	
N=91/393		Imatinib	imatinib+2GTKs	Imatinib	imatinib+2ITKs
	P=0,045	0,812	0,673	0,844	0,694
Sokal	Low 27/144(19%)	15/27(55,5%)	24/27(88,9%)	10/26(38,5%)	20/27(74%)
	Inter 40/182(22%)	23/37(62,1%)	31/37(83,8%)	15/38(39,4%)	29/40(72,5%)
	High 17/47(36%)	7/13(53,9%)	11/14(78,6%)	5/16(31,2%)	10/16(62,5%)
	P=0,0001	0,138	0,264	0,654	0,423
Euro	Low 28/175(16%)	13/28(46,4%)	23/28(82,1%)	12/28(42,9%)	21/29(72,4%)
	Inter 43/178(24%)	27/40(67,5%)	36/40(90%)	15/41(36,6%)	32/43(74,4%)
	High 12/20(60%)	3/9(33,3%)	7/10(70%)	3/11(27,3%)	6/11(54,5%)
	P=0,132	0,550	0,711	0,187	0,140
Eutos	Low 70/311(23%)	39/65(60%)	55/65(84,6%)	28/67(41,8%)	51/70(72,8%)
	High 11/32(34%)	5/10(50%)	8/10(80%)	2/10(20%)	5/10(50%)
ALL	91/393(23%)	48/84(58,3%)	71/85(83,5%)	34/87(39,1%)	65/90(72%)

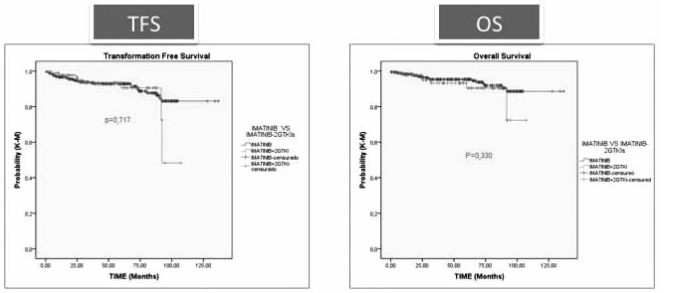

Figure 1.

Aims. Evaluate the actual benefit of 2G TKIs as salvage therapy for CML patients failing to Imatinib, regardless of the indication for its use, outside of clinical trials.**Study groups and Methods.** We have studied 393 CML patients, newly diagnosed, treated with imatinib as first TKI in our registry, which includes only patients outside clinical trials (compassionate uses are not excluded). These patients have been classified according whether second-generation TKIs were available or not. Group 1 includes 303 patients treated with imatinib or increased imatinib dose. Group 2 includes 91 patients (23%) that need treatment change (20 due to intolerance and 70 due to either failure, suboptimal response or loss of response to imatinib). 2G TKI prescribed were dasatinib in 43 patients, nilotinib in 25 patients and 23 patients received either dasatinib or nilotinib as third line after failure 2G TKI. **Results.** The use of second-generation TKIs as salvage therapy resulted in significant benefit to patients in terms of responses. Proportions of response (complete cytogenetic responses (CCR) at any time and major molecular responses (MMR)) only with imatinib and before 2GTKIs are included in Figure 1, according to Sokal, Euro and Eutos index risk groups. All groups of patients improve the rate of CCyR or MMR and the difference between groups of risk lost statistically significance. The outcome in terms of transformation free survival and overall survival was similar in patients with only imatinib (optimal response) and in patients that have to change to a 2GTKI (Figure1). **Conclusions.** The use of second-generation TKIs as salvage therapy has improved the responses of CML patients treated with TKIs. Outside clinical trials, the outcome of patients with 2GTKIs as salvage therapy seem to be similar that patients with good responses to imatinib. Although the use of 2GTKI frontline has shown benefit compared to imatinib, this therapeutic strategy merits, in our view, to be compared with the use of imatinib followed of 2GTKI for patients without optimal responses to imatinib.

1323

EVALUATION OF REAGENTS FOR THE QUANTITATIVE MULTIPLEX MEASUREMENT OF BCR-ABL1 EXPRESSION ON THE INTERNATIONAL SCALE

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Background. BCR-ABL1 e13a2 or e14a2 fusion transcripts corresponding to the major breakpoint M-BCR of t(9;22) are present in >95% of chronic myeloid leukemia (CML) patients. This molecular signature is useful to monitor tumor burden and TKI treatment response. Large studies have led to establishment of the International Scale (IS) for consistent interpretation between patients and laboratories. On this scale, baseline BCR-ABL1 (e13a2 or e14a2) to control gene ratio is defined as 100% IS with a 3-log reduction indicative of MMR (0. 1% IS or lower). **Aims.** The objective of this study was to evaluate the use of a multiplex assay (BCR/ABL1 Quant™ Test, CE-marked IVD) for the quantitative detection of e13a2 and e14a2 fusion transcripts. We investigated the Test's performance relative to a laboratory-developed test (LDT) reporting on the IS and the potential interference by rare fusion transcripts. **Methods.** A series of RNA specimens was generated by diluting cultured, immortalized cells in non-leukemic donor specimens. Purified total RNA, at an input optimized for the Test, was then reverse transcribed and amplified by multiplex, real-time quantitative PCR on the ABI 7500 Fast Dx (in "standard"mode) using target-specific primers for BCR-ABL1 (e13a2, e14a2, and e1a2) and ABL1 as an endogenous internal control. The variant e1a2 resulting from the minor breakpoint m-BCR was not evaluated in this study. Four-point standard curves were generated in each run using the included multiplex Armored RNA Quant® (ARQ) Calibrators. Each Calibrator, containing BCR-ABL1, ABL1, and BCR/ABL1 Quant Norm, an exogenous internal control, was prepared by heat lysis prior to reverse transcription. Data were analyzed using the 7500 Fast Dx instrument's software (SDS v1. 4). **Results.** Linearity of 5 logs was observed with the RNA specimens derived from a mixture of cultured leukemic cells in a background of non-leukemic cells (R2 > 0. 99). Known negative specimens did not generate a fluorescence signal. The breakpoints e13a2 and e14a2 were specifically detected. Inter-run reproducibility was excellent. An IS conversion factor of about 0. 2 was calculated using 37 residual clinical specimens tested in singleton across two runs. After conversion to the IS, 97% of the IS % ratios generated with the Test were within 3-fold of the LDT results and 81% within 2-fold. Specimens positive for the rare e19a2 fusion transcript (an exon e1-containing variant resulting from the micro breakpoint µ-BCR) yielded percent ratios on average 50-fold lower with the Test relative to the LDT. Specimens with the rare variants e13a3 and e14a3 were confirmed negative with the Test. **Conclusions.** The BCR/ABL1 Quant Test is compatible with reporting on the IS and provides results across a broad linear range. As expected, rare transcripts do not interfere with the Test. The Test does quantify e13a2 and e14a2 fusion transcripts in a single reaction and shows excellent correlation with the IS. Streamlined reagent formulation, multiplex assay format, and inclusion of ARQ calibrators improve the workflow and increase the number of specimens that can be tested per run.

1324

METHYLATION STATUS ANALYSIS OF THE ZONULA OCCLUDENS-1 (ZO-1) GENE PROMOTER REGION OF CHRONIC AND BLASTIC PHASE CHRONIC MYELOID LEUKEMIA

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Background. Despite encouraging treatment success has been obtained in chronic phase (CP) chronic myeloid leukemia (CML), the treatment in blastic phase (BP) remains a difficult problem. Exploring the markers of disease progression constitutes the important problem. Zonula occludens-1 (ZO-1) is one kind of membrane-associated guanylate kinases, previous study has showed the aberrant hypermethylation in ZO-1 gene promoter region may concern with leukemia pathogenesis and progression. **Aims.** The aim of this study was to compare the methylation status of ZO-1 gene promoter region between CP and BP CML patients and analyze the relationship between the methylation status and the disease progression. **Methods.** From March 2011 through December 2011, the methylation status of the ZO-1 gene promoter region of the bone marrow samples of 20 CML patients (10 CP patients and10 BP patients) was detect with Methylation-specific PCR (MS-PCR). The appropriate informed consent from each individual was obtained after explaining the nature and consequences of the research. **Results.** For the 10 CP-CML patients, there was no one showed hypermethylation in the ZO-1 ene promoter region, but for the 10 BP-CML patients, 7 patients showed hypermethylation. For one CP patient, the methylation status was changed from hypomethylation to hypermethylation when the disease advanced to BP, and was change back to hypomethylation when the diseases returned to CP after treatment. **Conclusions.** Our results showed that the methylation status of ZO-1gene promoter regions were significant difference between chronic and blastic phase of CML and its change may be closely related to disease progression.

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ACHIEVEMENT OF MAJOR MOLECULAR RESPONSE IN CHRONIC PHASE CML PATIENTS IN THE RUSSIAN FEDERATION TREATED WITH IMATINIB

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Background. Chronic Myeloid Leukemia (CML) is a type of myeloproliferative disease characterized by the chromosomal translocation between the part of the BCR gene from chromosome 22 and ABL gene from chromosome 9. As a consequence of this reciprocal translocation is a novel BCR/ABL gene coding a constitutively active protein, tyrosine kinase that activates multiple downstream signaling pathways. Activity of this fusion BCR/ABL protein can be specifically inhibited by tyrosine kinase inhibitor (TKI) - imanitib mesylate. According to clinical trials, patients who had MMR or those who have at least 3 log reduction of BCR/ABL transcript level after 18 months of imanitib therapy had a significantly lower risk of disease progression and better event-free survival than patients without major molecular response (MMR) at this time point [M. Baccarani et al, 2006[1]]. First use of imatinib in CML patients was started in Russia in 2001 and it became widespread since 2005. To date Russian Federation registry of CML patients includes 5866 patients from all the 83 federal subjects of Russia. **Aims**. To analyze proportion of patients who have achieved an MMR after 18 months of imanitib therapy of CML patients in chronic phase (CP) in the Russian Federation. **Methods.** We analyzed peripheral blood samples of 2215 CML CP patients from all of 83 federal subjects of the Russian Federation. All patients were on imatinib therapy (400-600 mg/day) more than 18 months. Relative BCR/ABL gene expression was measured by quantitative real-time polymerase chain reaction (RQ-PCR) in comparing with expression of reference gene ABL. Quantity of reference gene was no less than 10000 copies per reaction, so the sensitivity of RQ-PCR was 0. 01%. Relative expression of BCR/ABL gene was multiplied to conversion factors in order to obtain results according to international scale (IS). **Results.** In November of 2011, eleven laboratories of the Russian Federation working in the field of CML diagnostics successfully passed the international standardization for RQ-PCR for BCR/ABL gene expression according to IS. Since that time, BCR/ABL gene expression has been analyzed for 2215 patients. We estimated that frequency of MMR was 57. 7% after 18 months of imanitib therapy in chronic phase (CP) of CML patients in the Russian Federation (from 38. 9% to 66. 7% in various federal subjects). **Conclusions.** Tyrosine kinase inhibitors (TKIs) efficiently decrease BCR/ABL gene expression and successfully induce high rate of MMR in CP of CML after 18 months of therapy. We showed that MMR could be achieved for about half of patients (38. 9-66. 7%) and in this group imanitib therapy is reasonable for further treatment. However, the remnant part of the patient has no MMR, thereby their therapy should be re-assessed to second-generation TKIs to induce durable remission and safety of progression. Reference: [1]M. Baccarani, F. Guilhot, R. A. Larson, S. G. O'Brien, B. J. Druker. ASH Annual Meeting Abstracts 2006 108: 2138.

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IMPACT OF TREATMENT ADMINISTRATION ON SELECTION OF BCR-ABL1 KINASE DOMAIN MUTATIONS

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Background. Availability of different tyrosine kinase inhibitors (TKIs) with distinct anti-leukemic potency enables the optimization of current therapeutic regimens; however, some patients lose their therapy responses and acquire TKIs resistance. **Aims.** In this study, a single center experience with monitoring of *BCR-ABL1* kinase domain (KD) mutations is described and, particularly, the impact of the treatment administration on mutations selection is discussed. **Methods.** The CML patients treated with TKIs at our institution during the years 2003 - 2011 were included in this study. Peripheral blood and/or bone marrow samples were collected, processed and analyzed using the published procedures; detection of *BCR-ABL1* KD mutations was performed by direct sequencing. **Results.** First, we evaluated the impact of non-TKI pretreatment on: a) spectrum of *BCR-ABL1* KD mutations; b) their frequencies and c) time to mutation detection after the administration of a first-line TKI. Our data shows that pretreatment with non-specific non-TKI drugs does not preferentially select the *BCR-ABL1* KD mutations, as there was no difference between the frequency of T315I or p-loop mutations compared to mutations in other KD regions (52. 7% vs. 47. 3%). In contrast, the imatinib (IMA) as first-line therapy led to clear predominance of T315I or p-loop mutations (90. 9% vs. 18. 2% of mutations located in other KD regions). In addition, median time to the detected p-loop mutations was substantially shorter in patients treated with IMA first-line compared to patients who were pretreated (16 months (3 - 41) vs. 46 months (20 - 109), p = 0. 005). Second, we analyzed the impact of administered TKIs on appearance and selection of mutations in patients that were recurrently resistant to TKIs therapy. Among the patients with initial *BCR-ABL1* KD mutation, 32. 0% developed a second mutations after the change of TKIs treatment. The more potent second-line TKIs therapy caused the elimination of most of the initial IMA resistant mutations; however, several mutations resistant to second-line therapy subsequently emerged, with considerably shorter time compared to the initially detected ones (9 months (1 - 25) vs. 21 months (3 - 109), p = 0. 007). Moreover, after the therapeutic intervention with third-line TKIs, similar consequences were observed. Third, we confirm the previously described poor prognosis of CML patients with mutated *BCR-ABL1* KD, since 40. 0% of our CML patients that harbored *BCR-ABL1* KD mutation died during the treatment with TKIs. Moreover, 27. 8% of patients who are still on treatment have already progressed. Last, our data confirms the unique position of T315I mutation with respect to its strong resistance to the currently approved TKIs. **Conclusions.** Based on a "real-life"data described in this study, for some of the CML patients that harbor *BCR-ABL1* KD mutations, it may seem that therapy itself consecutively leads to its failure, selecting thus the most resistant mutations under the selective pressure of applied therapy regimen (Figure 1). Note that formation

and selection of mutations, that could reflect the genomic instability, is not a single process, thus all aspects must be taken into account. **Acknowledgements.** Supported by The *CzE*ch *L*eukemia Study Group for *L*ife (*CELL*) and grants MSM0021622430 and MUNI/A/0784/2011.

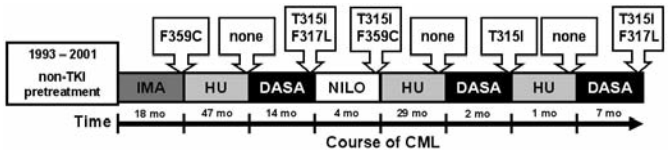

Figure 1. CML patient - a therapy-driven selection of BCR-ABL1 KD mutations; mo - months (IMA - imatinib, DASA - dasatinib, NILO - nilotinib, HU - hydroxyurea).

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EARLY AND DEEP RESPONSES TO IMATINIB ARE STRONG PREDICTORS OF COMPLETE MOLECULAR RESPONSE

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Background. Imatinib has consistently proven a survival benefit in patients with chronic myeloid leukemia (CML). Second line tirosine kinase inhibitors (TKI) seem to bring deeper and earlier responses, but to asure that this means survival benefits requires longer follow up. TKI need to be taken for unlimited period of time in order to avoid the risk of accelerated phases. It has been described recently that patients with a continued, complete molecular response (CMR) for at least 2 years, could safely quit treatment. Less is known about the real rates of patients under TKI to achieve CMR and which factors may add to maintain the response. **Aims.** To analyze the probability of achieving CMR of patients treated with imatinib in the long term follow up and to identify factors predicting its achievement and maintainance. **Patients and Methods.** We have analyzed 108 patients treated with imatinib as first tirosine kinase inhibitor in a single institute. Median follow-up was 55 months (10-124). Sex ratio (M/F) was 54/46 with a median age of 53. 1 years (27. 4-78. 2). Sokal score distribution was 42%, 40% and 18% for low, intermediate and high values respectively. Eutos score distribution was 87% and 13% for low and high values. 48% of the pts received interferon therapy prior to imatinib. We used European Leukemia Net recomendations for the follow up. CMR was defined as $CMR^{4.5}$: *BCR-ABL/ABL IS ratio* <0. 0032% or UMRD (undetectable Bcr-Abl using standardized RTQ-PCR) under imatinib therapy in two consecutive determinations. **Results.** The probability of a CMR at any time during treatment was 38% (N=42). Only 22 of the 42 patients (20% in the entire cohort) that achieved CMR mantained it for a period of 2 years. We have indentified the cytogenetic responses at 6 months and the molecular responses at 12 months as strong predictor factors for CMR. Probability for CMR during follow up was 63% for patients with complete cytogenetic responses (CCyR) at 6 months vs 17% for patients that did not get a CCyR at that time (p=0. 00). Patients with mayor molecular response (MMR) (<0. $1\%^{IS}$) at 12 months showed more probabilities for achieving CMR (81% vs 24% p=0. 000). Neither Sokal or Eutos prognostic factors were identified as predictor factors for CMR. **Conclusions.** Complete molecular responses maintained for 24 months are not so frecuent in patients under imatinib. We have identified early and deep responses as strong predictor factors for this achievement. Strategies that improve cytogenetic and molecular responses, as the use of a second line TKI, could increase CMR rates, as well as the possibilities for a discontinuation of the treatment.

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THE Results OF 10-YEAR TREATMENT PERIOD BY TYROSINE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN LATE CHRONIC PHASE

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Background. Imatinib (IM) therapy in chronic myeloid leukemia (CML) patients started in Russian Federation from 2000-2001. The first patients treated by IM had a late chronic phase (LCP) with IM therapy started in >6 months from diagnosis. **Aims and Methods.** To analyze the results of the IM treatment in the LCP CML patients within the durable follow-up period. 219 LCP CML patients (107 males and 112 females) who started IM from 2000 to 2004 were analyzed**.** Median age at diagnosis was 41 years (9-73years). Median period from diagnosis till the data assesement was 116 months (16-165 months). Median CML duration from diagnosis till IM treatment was 32 months (6,5 -57 months). Sokal risk groups ratio was 143(65%):32(15%):44(20%) for low, intermediate and high risk correspondently. 192(88%) of patients received interferon alpha (IFNά) before IM treatment, 29 (13%) received busulfan. Median duration of IM treatment was 79 months (0,9 - 118 months), median duration of the following treatment by 2nd generation of tyrosine kinase inhibitors (TKI2) in 60 patients was 40 months (2-61 months). **Results.** The nine years overall survival (OS) for LCP CML patients from IM treatment start was 78% and significantly differed in low, intermediate and high Sokal risk groups: 84%, 75% and 61% correspondently (p<0,002). At the moment of evaluation 171 (78%) patients were alive and on TKI treatment: IM in 121 (55%), mostly on standart dose 400 mg/day and TKI2 in 48(23%) of patients; 2 patients resistant to IM and TKI2 receive hydroxyurea. The stable complete cytogenetic response (CCyR) was achieved in 147(67%) of patients; in majority of cases during first 3 years of treatment, in some patients CCyR was achieved firstly on 4th-8th year of treatment. 93 (75%) of the evaluated patients achieved a major molecular response (MMR), 47 of them also got a complete molecular response (CMR), in 10 of patients MMR was not stable. 31 (25%) of the evaluated 124 patients did not get MMR. Progression to accelerated phase (AP) or blast crisis (BC) developed in 74(26%) of patients in median period of 71 months (7-86 months). 48 (22%) patients died: 41(85%) of them from disease progression, 5 (11%) from comorbidities, 2 (4%) from unknown reason. OS in patients with CML duration >60 months before IM treatment, 12-60 months and 6-12 months significally differed: 60%, 84% and 83% correspondently (p<0,02). Usage of busulfan before IM had a negative impact on OS: OS was 81% in patients without busulfan pretreatment vs 59% in those who received busulfan (p<0,001). The highest mortality in patients pretreated by busulfan was within the first 3 years of IM treatment, and they got CCyR in later terms. The group with IFNά pretreatment had significantly higher OS compared to the group without IFNά pretreatment: 80% vs 60%(p<0,001) correspondently. **Conclusions.** TKI therapy in LCP CML patients permitted to restore the Ph'-negative hemopoesis even on late terms of therapy and also allowed to achieve the high rates of OS and long-lasting response rates in group of CML patients with the durable pretreatment period.

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CLONAL EVOLUTION IN PHILADELPHIA CHROMOSOME-NEGATIVE CELLS DURING IMATINIB MESYLATE THERAPY IN PHILADELPHIA CHROMOSOME POSITIVE CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. Imatinib is current standard treatment for newly diagnosed patients (pts) with CML. Secondary chromosomal changes are known to develop in Philadelphia chromosome negative (Ph-) cells in chronic myeloid leukemia (CML) during treatment with imatinib. The most common abnormalities are trisomy 8 and monosomy 7 occuring in 3-4% patient with CML. **Aims.** The aim of our study was to investigate chromosomal changes in Philadelphia negative cells in large cohort of our CML pts. **Patients.** Between 2002 and 2011, 140 pts with CML were treated with imatinib in our institution (frontline in 102 and 38 pts as second line). Imatinib therapy was in standard dose 400 mg, with escalation to 800 mg/d in patients not achieving optimal response according to ELN and national guidelines. **Results.** In our group, 9 patients (6. 4%), 4 males and 5 females, mean age 38 years (27-79 yrs) developed clonal chromosomal abnormalities in Ph-negative cells. They were noted on 14 months in average (range, 6-26) after imatinib therapy was initiated. All pts were in chronic phase CML, 3 pts had low risk, 4 pts intermediate and 2 pts high risk Sokal score and all patients achieved complete cytogenetic response (CCgR) at six months. Six patients had trisomy 8 and three lost chromosome Y without myelodisplasia. The median interval of the first trisomy 8 observation was 12 months (range 6-24). Three of six patients with +8 lost cytogenic response after 24, 36 and 38 months from start of imatinib. They were subsequently treated with Nilotinib (2 pts) or escalated dose of Imatinib (1 pts) and all pts achieved again CCgR and also complete or major molecular response (MR3. 0/MR4. 0) without progression during further follow-up of 26 months (range 6-64 months). In all pts with the loss of chromosome Y, with median follow-up of 24 months (range 6-48), no progression of CML was observed. **Conclusions.** Imatinib may favor the emergence of additional clonal chromosomal abnormalities. Those abnormalities in Ph-negative clone were not associated with myelodisplasia. These

changes did not impair the cytogenetic response to IM but in some patients may lead to loss of prevoius treatment response. In general, imatinib therapy was not interrupted due to those aberrations. Futhermore, our current findings suggested that patients on imatinib should be regularly monitored by all means like cytology, and conventional karyotype besides regular molecular monitoring.

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SUDDEN BLASTIC TRANSFORMATION AFTER A GOOD RESPONSE WITH IMATINIB IN CML PATIENTS, EXCEPTION RATHER THAN THE NORM

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The introduction of tyrosine kinase inhibitors (TKIs) has proved to be a major advancement in the management of patients with chronic myeloid leukemia in chronic phase (CML-CP)[1] although the clinical benefit seems to be limited to those patients who achieve CCyR. Patients who achieve CCyR are believed to have an excellent prognosis, however a very small proportion of those patients may loose the response and progress to blastic phase. In this work we study a cohort of 210 patients who achieved CCyR on imatinib first line therapy in order to identify the incidence of blastic transformation in this population. Between June 2000 and December 2010, 210 consecutive adult patients with CML-CP received imatinib 400 mg daily as first-line therapy (as described earlier[2]) at our institution. The median follow-up was 69 months. Of the 210 patients, 5(2. 3%) progressed to blastic phase (3 myeloid and 2 lymphoid). The median time to achievement of CCyR in these 5 patients was 18 months (range 9-20 months). Four patients achieved a major molecular response (MMR, one of them achieved a 4 log reduction in the transcript levels) and one patient had a CCyR with no MMR. None of the patients had a mutation prior to transformation to an advanced phase and subsequently two patients developed a new mutation at the time of transformation (M244b and T315I). The cumulative incidence (CI) of blastic transformation after attainment of CCyR was 2. 3% (Figure 1). The median time from achievement of CCyR to blastic transformation was 18 months (Range 12-40 months). In summary, sudden blastic transformation is a rare but devastating event in patients who have achieved CCyR on imatinib with an 8 year CI of 2. 3%.

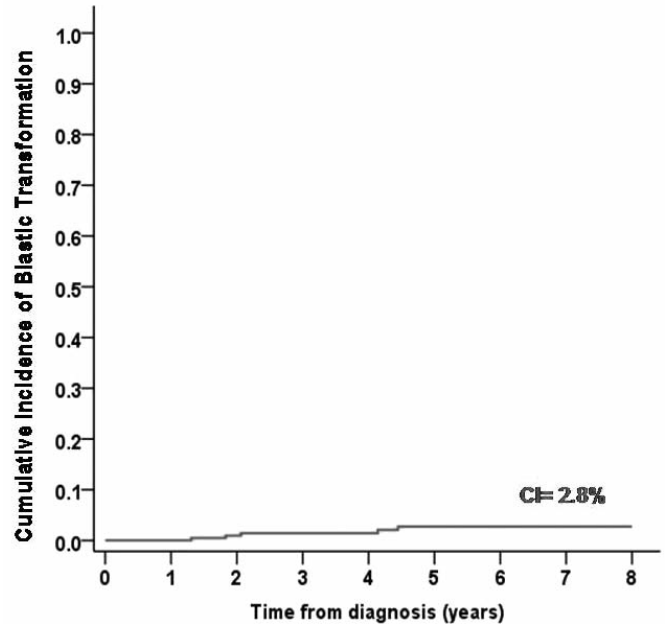

Figure 1. CI of progression to blast phase after attainment of CCyR, n=210.

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CHRONIC MYELOGENOUS LEUKEMIA IN LEBANESE AND JORDANIAN CHILDREN AND ADOLESCENTS

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Background. Chronic myelogenous leukemia (CML) is rare in childhood. Allogeneic hematopoietic stem cell transplantation (AHSCT) remains the only proven cure. However, the excellent outcome induced with the advent of molecularly-directed therapy that inhibits the tyrosine kinase (tyrosine kinase inhibitors, TKI) in adults resulted in a radical change in the management of pediatric CML. **Aims.** We report our joint experience with pediatric CML at the Children's Cancer Center of Lebanon (CCCL) and King Hussein Cancer Center (KHCC) in Jordan and the associated challenges. Patients and **Methods.** We retrospectively reviewed data on children and adolescents younger than 20 years, who were diagnosed with CML for the period of April 2002 through May 2011. An informed consent was obtained from either the patients or their legal guardians. Medical records and laboratory data were reviewed for clinical presentations, disease course, response to treatment, adverse effects and tolerance. **Results.** A total of 17 children (7 from CCCL and 10 from KHCC) with CML were identified. There were 10 males (58. 8%) versus 7 females (41. 2%) with a median age at diagnosis of 10. 5 years (range, 1 to 20 years). The median follow-up time was 4. 5 years (range, 1 to 12 years). A complete hematologic response was achieved initially in all of patients on imatinib mesylate. An optimal response (complete molecular response after 18 months and complete cytogenetic response at 1 year) was achieved in 76. 4% (13/17 patients) of cases. The median time to achieve complete molecular response was 9 months (Figure 1). Two patients failed imatinib and were switched to second generation TKI (one achieved complete molecular and cytogenetic response while the other awaits AHSCT) and 4 underwent AHSCT. None of the patients died. There were no major adverse effects reported, particularly those related to delayed growth velocity in children. However, the major challenge with TKI therapy was compliance, especially in adolescents. **Conclusions.** Imatinib mesylate has become the first-line of treatment in children with CML with AHSCT reserved to those who have full-matched related donors or fail medical treatment. On the other hand, further studies are needed to assess the efficacy of second generation TKI in children. The major challenge to medical treatment in our series was the availability of the drug in developing countries and the adherence to therapy, especially in adolescents.

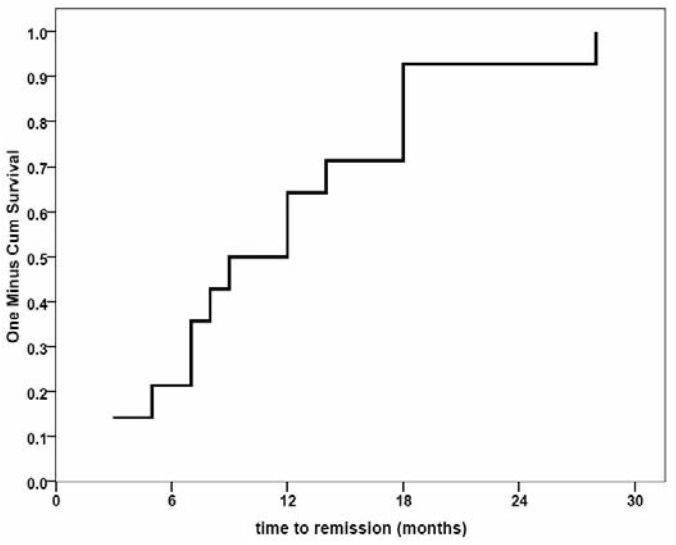

Figure 1.

1332

A RETROSPECTIVE REAL-LIFE ANALYSIS OF CHRONIC MYELOID LEUKEMIA PATIENTS IN SUBOPTIMAL RESPONSE TO IMATINIB: A SINGLE CENTRE EXPERIENCE

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Background. Life expectancy of CML patients has greatly improved in tyrosine-kynase inhibitor (TKI) era, but still some questions remain about the management of suboptimal responders (SR) to imatinib standard dose. European Leulemia Net (ELN) recommended to continue imatinib in case of optimal response and to move to second generation TKI in case of failure but there isn't a clear agreement on SR: maintaining imatinib at standard or higher dose or switching to another TKI are all considered acceptable options (Baccarani et al, JCO 2009). **Aims.** Outcome evaluation of 43 SR patients to imatinib 400 mg/d treated according to the 3 different ELN options. **Methods and Results.** We retrospectively analysed 43 CML patients, diagnosed in chronic phase between 1988 and 2011, SR to imatinib 400 mg/d. Forty patients received imatinib front line, 3 had been previously treated with interferon and cytarabine. Sokal score, evaluable in 27 patients, was high risk in 2, intermediate in 13 and low in 12, respectively. Seventeen patients were cytogenetic SR and 26 molecular SR according to 2009-ELN. The median follow-up from diagnosis was 72 months (range 10-288), only one patient died (gastric cancer). At suboptimal response detection 33 patients (77%) continued imatinib 400 mg/d, 9 increased imatinib dose to 600 mg/d (4) or 800mg/d (5), one switched to new TKI (response not yet evaluable). Among the patients who continued imatinib 400 mg/d (88% for at least 6 months), 9 (27%) major molecular responses (MMR) (7 long-lasting) and 13 failures (39%) were detected. Ten patients maintained a suboptimal response and eventually increased imatinib dose (9/10) or changed TKI (1/10). Eighteen SR patients increased imatinib dose, 50% at suboptimal response detection and 50% after further 12 months (median) of standard dose treatment (range 3-18). Thirteen patients (72%) obtained MMR, that was durable in 11 (61%). Four failures were observed (one after the achievement of MMR, one after intolerance to high dose imatinib). A total of 12 SR patients switched to a new TKI (8 dasatinib and 4 nilotinib), 10 after high dose imatinib, and 5 of 8 evaluables (62%) achieved MMR. At the end of the follow-up 32 patients (74%: 59% of the cytogenetic SR and 85% of the molecular SR) were in stable MMR; 6 more patients achieved complete cytogenetic response only. No patient progressed to accelerate/blastic phase. **Conclusions.** In our casistics no clear advantage in maintaining imatinib 400 mg/d after suboptimal response was observed (only 21% durable MMR, 39% failures). Imatinib dose increment led to a 61% of stable MMR and might represent a more reasonable option. The switch to 2nd generation TKI brought results comparable to those obtained with high dose imatinib (62% MMR) in patients mostly unresponsive to the last option. Therefore, an earlier switch to new TKI might further increase the proportion of optimal responders. However, the small number of patients treated with new TKI in our study prevents definitive Conclusions about the optimal management of SR.

1333

IS THERE A DIFFERENCE IN TREATMENT OUTCOMES OF ADOLESCENTS&YOUNG ADULTS OR ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA?

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In leukemia treatment, most of treatment efforts are directed towards improving outcomes in younger patients providing better long term survival. On the contrary, CML is disease of advanced age, and often in clinical practice certain number of patients >60y would not receive TKI treatment due to comorbidities, possible drug interactions or clinical judgment. Also, it is known that Sokal score is rising with patient's age and in general, elderly population had worse outcome in classical CML. **Aims.** We analyze outcomes of frontline imatinib treatment in large cohort of CML patients from database of Clinical Center of Serbia from 2005 to 2011. **Patients.** In last 7y, 102 patients with CML were treated by imatinib frontline. Outcome was assessed as optimal response on 6 and 12 months of treatment according to ELN proposals. **Results.** Patients were separated in 4 age groups (younger ones 15-29y, middle age 30-44y and 45-60y, and elderly patients with age >60y). Mean age in the whole group was 54y (16-85). Younger group had 13 pts, while other groups had 21, 38 and 30 pts respectively. At 6 months, in young group optimal response (majorCgR) was achieved in 11 of 13 patients (84%), but CCgR was achieved in 7 pts (53%). On the other side, in elderly group, majorCgR was achieved also in 82% of pts (23/28), but CCgR was achieved in 19/28 pts (67%) (Fisher p=0. 29). At 12 months, in young group optimal response (CCgR) was present in 7 of 11 patients (63%). On the other side, in elderly group, CCgR was achieved in 22 of 25 pts (88%) (Fisher p=0. 08). We have analyzed other variables that might influence obseved differences and we have found that younger patients have more often high WBC count (180 vs. 77. 6 G/L, t test p<0. 01), higher blast count in blood (2. 1% vs. 0. 8%, t test p<0. 05) and also larger spleens (6. 5 vs. 2. 4 cm below LCM, t test p<0. 05). Large splenomegaly (>5cm) was noted in half of younger patients 7/13 (54%) but only in 7 of 30 pts in elderly group (23%)(Chi sq p=0. 05, Fisher p=0. 055). We have not found differences in basophil, eosinophil or platelet count. We have found that there was a difference in Hasford score distribution between these two groups, younger ones were more low risk (8/13), and elderly were intermediate risk (22/30) (Chi square p<0. 05). There was no difference in EUTOS score between two groups. Two younger patients died (15%) one due to blastic transformation and other one during SCT, and 3 elderly patients died (10%), 2 in blastic transformation. Overall survival of those two distinct groups was similar, OS5y 92% for younger and 88% for elderly population of patients. **Conclusions.** Our results are in concordance with recently published data suggesting that young adults have less optimal outcome on long term, therefore they need different prognostic and therapeutic approach. Also, we have found that most of patients >60y can tolerate imatinib treatment well with achievement of excellent treatment goals like CCgR.

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DYNAMIC OF CML INCIDENCE AND PREVALENCE IN SAINT-PETERSBURG AND LENINGRAD REGION

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Background. Tyrosine kinase inhibitors(TKI) dramatically change the life of patients with chronic myeloid leukemia(CML) - drastic decrease of disease progression and increase of the overall survival of CML patients. Evaluation of incidence and prevalence is important for planning of therapeutic and financial strategies in the region. **Aims.** To evaluate the dynamics of CML incidence and prevalence in Saint-Petersburg and Leningrad region. **Methods.** CML patient (pts) databases were obtained from all hematological clinics and outpatient departments of Saint-Petersburg(SPB)and Leningrad region(LR). In 2005 the data of all alive or ever treated with Imatinib (IM) CML pts (Ph and/or bcr-abl positive) were collected. Afterwards all new CML cases were registered and updated at least annually. To evaluate the incidence and prevalence we used the data of the general census of the population of Russia in 2010(SPB population - 4848700 and LR -1712700). The data were analyzed using SPSS statistics 17. 0. **Results.** In the database there are data of 477 CML pts: 56, 170 and 251 pts of them were diagnosed before 2000, during 2000-2005 and 2006-2011 respectively. The most of them (347/477, 73%) are alive at the time of analysis. The mean incidence of CML during 2006-2011 was 0,64 (range 0,44-0,8) with median age of 53,2 years. The mean incidence of male/female CML ratio was 0,7:0,6. At the diagnosis the mean annual frequency of chronic phase (CP), accelerated phase (AP) and blastic phase (BP) was 93%, 5% and 2% respectively. In the whole group TKI therapy was ever given to 438/477(91,8%) pts (IM was ever given to 427 pts). The majority of pts (245/251, 97%) diagnosed during 2006-2011 was treated with TKIs as first line (IM in 234, other TKIs in 11pts), whereas the pts diagnosed before 2006 (151/193, 78%) mainly obtained IM in late CP. Second generation TKI were given to 90/427 (21%) IM resistant or intolerant pts. In the database there are only 16 pts after alloSCT (5 of them are alive, 10 - dead and 1 - lost to follow-up). At present the quantity of pts alive and the prevalence of CML nearly doubled as compared with the end of 2005 (Table 1). Estimated overall survival by 8 year for pts treated with

IM in early and late CP was 90% and 73% (p=0,028). Estimated overall survival was significantly lower in patients with high versus low Sokal score irrespective of IM start time (early or late CP, p=0,014 and 0,000). Whereas the estimated overall survival in low and intermediate risk score groups was equal only in pts who received IM in early (p=0,12), but not in late CP(p=0,009). **Summary.** In TKI era significant increase of CML prevalence is documented in our region. Overall survival of pts with low and even intermediate Sokal score is very impressive, meanwhile pts with high Sokal score are still at considerable risk of IM failure. Increasing number of pts with early start of IM and timely switch to new TKIs yield Results. overall survival during last years is notably increasing.

Table 1. The quantity of alive pts and CML prevalence in Saint-Petersburg and Leningrad region during 2005-2011 years.

Parameter	At the end of year						
	2005	2006	2007	2008	2009	2010	2011
Quantity of pts(n=)	187	207	247	255	292	310	347
CML prevalence	2,85	3,15	3,76	3,89	4,45	4,72	5,29

1335

SIGNIFICANCE OF MOLECULAR MONITORING IN CML PATIENTS WITH CCYR AFTER PCR INTERNATIONAL STANDARTIZATION IN ST-PETERSBURG AND LENINGRAD REGION

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Background. In tyrosine-kinase inhibitors era chronic myeloid leukemia (CML) is potentially curable disease. Due to close monitoring of the treatment effectiveness clinicians can make appropriate decisions in time. Nowadays molecular monitoring became available in many countries and due to international standardization of RQ-PCR methods in different laboratories results can be compared and interpreted according to international recommendations. **Aims.** To evaluate the achievement and stability of major molecular response (MMR) in CML chronic phase (CP) patients with complete cytogenetic response (CCyR) while Imatinib therapy. **Materials and Methods.** In this study, 153 pts with CML CP with the different date of diagnosis, older than 18 years, treated with imatinib, CCyR and at least 1 molecular analysis by international scale (IS) were included. During 2011 325 samples were analyzed. The median age of patients at the moment of diagnosis was 49. 85 yrs (18. 6-81. 9). Male/female ratio was 72/81. The qPCR testing was performed in standardized by IS in 2011 local laboratories (Federal Almazov center, Russian Research Institute of Hematology and Transfusiology). **Results.** The achievement of MMR in this group was 78% (119/153). The rate of MMR increased in time (Figure 1).

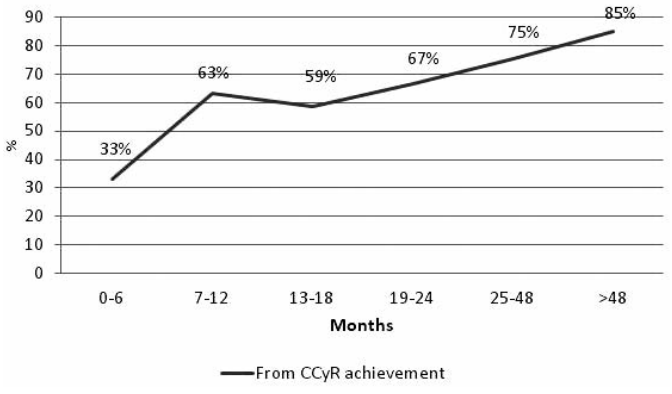

Figure 1. Dynamic of MMR rate according to the time from CCyR achievement.

During the evaluation we didn't reveal any significant factors, influencing on achievement of MMR (time to CCyR, MCyR, time from the diagnosis to imatinib start, Sokal score). The loss of MMR was 7,8% (8/103). In 3 cases MMR was reobtained without any treatment changes. No one patient with MMR subsequently lost CCyR, whereas 1 patient without MMR did. No one patient progressed to accelerated phase and blast crisis or dead during observational time. **Conclusions.** The probability of MMR achievement increases in time. Achievement of molecular response as well as CCyR gives high probability of progression-free and overall survival. Molecular monitoring provide early detection of response loss.

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EFFICACY OF NILOTINIB IN EARLY CHRONIC PHASE CML PATIENTS WHO HAVE SUBOPTIMAL CYTOGENETIC OR MOLECULAR RESPONSE TO IMATINIB. A MULTICENTRIC RETROSPECTIVE STUDY

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Backgroud. The CML-CP suboptimal responders rappresent an eterogenous group of patients because they can either obtain an optimal renponse or experiment a failure. The Clinical trials at MDACC,HammersmithHospitaland GIMEMA observed that patients with suboptimal response at 6 and 12 months have worse long term outcomes than patients with optimal responses, particularly if the suboptimal response occurs early in the treatment, suggesting an advantage for pts with early major molecular response expecially for event free survival and progression free survival. Recently, the German group showed the benefit of early major molecular response on overall survival too. So these kind of patients deserve to be switched to other treatments even if ELN does not recommend automatic change of therapy for them. **Aims**. This study was designed to explore the efficacy of the early switch to Nilotinib in patients with suboptimal responses to imatinib (IM) according to ELN. **Methods.** In this multicentric retrospective study, 12 CML-CP patients with suboptimal response to IM within 24 months from diagnosys were evaluated: 4pts with a low, 3 with intermedied and 5 with high Sokal score. The best response to IM was CCyR for6 pts, PCyR for one pt and Complete Hematological Response for5 pts. As for suboptimal responses,5 pts were defined in suboptimal cytogenetic response:2 pts at 12 months and2 pts at 6 months; 6pts were 18 months suboptimal molecular responders and1 pt had a loss of CCyR at 12 months. All patients were switched to Nilotinib 400 mg twice daily. Bone marrow was done at baseline in all pts and at 3,6,12 and 18 months in cytogenetic suboptimal pts until CCyR, while the molecular analysis was performed on peripheral blood every three months as for molecular suboptimal pts. **Results.** 12 pts have been treated with Nilotinib for a median of 17,5 months (range 3-37), 9 patients for ≥ 12 months. Before switching to Nilotinib pts were treated with IM 400 mg once daily apart for 2 patients who needed an adjustment dose to 300 mg and 600 mg for toxicity and suboptimal response, respectively. Among6 ptswith suboptimal CyR, 4 obtained CCyR, 3 at 3 months and one at 6 months;2 ptshad any response at the milestones timepoints and they switched to another therapy. All pts with molecular suboptimal response obtained MMR at 3 months apart for one, who showed MMR at 12 months. Nilotinib was well tolerated in all12 pts; only one developed a moderate transaminase elevation. A brief drug intrerruption was sufficient to manage this adverse event. **Conclusions.** Nilotinib treatment results in high and relatively quick cytogenetic and molecular response rate in CML -CP- pts with suboptimal response to IM. These preliminary results demonstrate that early switching to Nilotinib couldbe raccomanded in suboptimal responders in order to improve the outcome of this kind of pts. A larger patient population and a longer period of observation could confirm these preliminary data.

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ASSESSMENT OF CARDIAC FUNCTION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB AT THE NATIONAL CENTER OF HEMATOLOGY

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Background. Imatinib has transformed the outlook for chronic myeloid leukaemia (CML) and gastrointestinal stromal tumour**.** Although rare, severe

CHF and left ventricular dysfunction have been reported during imatinib treatment, especially in patients with risk factors or comorbidities. The mechanism underlying imatinib induced cardiac failure is currently unclear. In an in vitro study, physiological concentrations of imatinib significantly and adversely affected mitochondrial membrane potential, apoptosis, cell viability, and cellular ultrastructure. **Aims.** To evaluate the effect of imatinib therapy on the cardiac function in patients with CML. **Methods.** This is a cohort prospective study conducted at the National Hematology center of Almustansiriya university between May 2008 and December 2009, fifty sequential patients with CML included in this study . All patients received Imatinib (Gleevec®;Novartis Pharmaceuticals Corporation, New Jersey, USA) 400 mg/day. All patients gave informed consent. The criterion for entry into the study was treatment for CML with imatinib at any dose for at least 12 months. ECG and Echocardiography were used to evaluate the cardiac function. **Results.** Of the 50 patients evaluated, 24 (41%) were men, and 26 (59%) were women. Their ages ranged from 18 to 74 years, with a median age of 36. 8 years. The mean duration of imatinib treatment prior to enrolment was 3. 4±1. 8 years. there were no differences in ejection fraction before and after imatinib therapy P value >0. 5. **Conclusions.** the study confirm that imatinib has no negative effect on cardiac function in CML patients.

Table 1. Shows the relationship of Ejection Fraction (EF) with patients subgroup according to gender, age and duration of imatinib treatment.

factors	subgroup	No.of patients	Mean EF ±SD at start	Mean EF ±SD after 1 yr	P value
Age(yrs)	15-25	8	57.13±6.221	56.00±6.908	
	26-35	18	60.28±4.824	59.78±4.918	
	36-45	11	60.18±8.589	57.64±6.439	NS
	46-55	10	59.00±8.000	58.80±7.941	
	56-65	2	53.00±1.414	53.00±2.828	
	66-75	1	55.00	56.00	
gendre	male	23	59.09±6.815	58.70±7.245	NS
	female	27	59.11±6.565	57.70±5.254	
Imatinb Duration (yrs)	1	10	60.30±6.129	59.50±5.442	
	2	12	53.17±3.298	54.17±3.614	
	3	2	64.50±6.364	64.00±8.485	NS
	4	15	61.07±7.156	60.60±7.980	
	5	4	60.00±8.264	54.50±3.512	
	6	7	61.29±4.716	58.29±2.984	

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APPLICATION OF THE NEW MOLECULAR PROGNOSTIC RISK SCORE FOR MONITORING CML-CP PATIENTS IN ONE SINGLE INSTITUTION

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Background. The introduction of tirosin kinase inhibitors (TKIs) has proven to be a major advance in the therapeutic of Chronic Myeloid Leukemia in chronic phase (CML-CP). Development of molecular based assessment like R-PCR and RQ-PCR permitted a better diagnosis and control of therapy and defining a new disease level, molecular disease. Until now no prognostic factors in early-stage disease has been validated to predict poor response to TKIs; in January 2012 *Marin et al*. (JCO 2011 38. 6565) published a new prognostic score: molecular score (MS) based on molecular assessment (MA) during the first year of TKIs therapy. **Aims.** To describe demographic and clinic characteristics of CML patients who received TKIs as first-line therapy in our institution and applied the MS based in assessment of BCR-ABL1 transcript levels at 3, 6 or 12 months as *Marin et al* described. **Patients and Methods.** A review of clinical histories of patients treated with TKI as first-line therapy since December 1999 to February 2012 was performed. Data on clinical characteristics, date of first TKIs therapy, MA and outcomes were obtained. Molecular assays were made using RQ-PCR technology; results were expressed as percent ratios relative to a gen internal control. European Leukemianet criteria for response were applied. **Results.** A total of 37 adult patients have been diagnosed of CML-CP and received TKIs as first-line therapy in our department. Mean age 52. 4 years (28-72), M/F ratio 23/14. According to Sokal risk score: low: 20 (54. 05%), medium: 10 (27. 02%), high: 4 (10. 81%). For Hasford risk score: low: 27 (72. 97%), medium: 3 (8. 10), high: 4 (10. 81%), missing data occured in 3 patients. First-line therapy: 2 patients received nilotinib and 35 Imatinib. MA of BCR-ABL1transcripts level at 3 and/or 6 and/or 12 months were made in 33 (89. 2%) patients. According to *Marin et al.* molecular score: 4 patients (12. 12%) had poor molecular prognosis (≥ 9. 5% transcript levels at 3 months, and/or ≥ 1. 67% at 6 months, and/or ≥ 0. 53% at 12 months), one achieved good molecular response at 12 months; 29 patients (87. 88%) had good molecular prognosis. Actual status revealed: 5 patients (15. 15%) had a Suboptimal MR (soMR) (only one was classified as poor molecular responder); 28 (84. 65%) had MMR and 8 patients (24. 25%) achieved a CMR. CCyR was achieved in all cases before 12 months. One patient was classified into poor risk group at 3 months and lost response to Imatinib by T315I mutation 3 years later. One patient discontinues Imatinib therapy to start on fertility program. ITK change: 6 patients switched to dasatinib because of intolerance and 1 for soMR; 2 patient switched to nilotinib, one because of intolerance and one for soMR The rest of patients (27) continue with Imatinib, 96. 29% in MMR, 22. 22% in CMR and 3. 70% (1) in soMR. No progression neither deaths were registered. **Conclusions.** Despite our small experience, application of molecular response risk score could be useful for predicting response CML-CP patients; more studies are required to determine the correct management approach specially in the poor risk patient group.

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CLONAL EVOLUTION IN PHILADELPHIA CHROMOSOME NEGATIVE CLONE IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS TREATED WITH IMATINIB, CYTOGENETIC FINDING AND CLINICAL COURSE

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Background. Imatinib has set a new standard of treatment outcome in patients with chronic myeloid leukemia (CML). However, not all of the patients get long term disease free status. Besides transformation led by additional mutation and clonal evolution (CE) in Philadelphia chromosome (Ph) positive leukemic clone, CE in Ph negative clone have been frequently observed. **Methods.** Data were collected from patients diagnosed with CML who had Ph chromosome as sole abnormality during 2001 through 2006. All of them were treated with 400 mg/day of imatinib. Cytogenetic study were performed intervally according to ELN guideline. Clinical finding, marrow finding and cytogenetic results were analyzed by descriptive basis. **Results.** CE in Ph negative clone has been found in 13 of 104 patients treated with imatinib (12. 5%). The median onset of CE was 12 months after initiation of imatinib (range 9 to 57 months). Additional chromsome 8 is the most frequent cytogenetic abnormality, followed by deletion of chromosome 7. Marrow examination from all of the patients did not display dysplastic feature. The finding of CE in Ph negative clone did not lead to treatment modification in our patients and no disease transformation was observed. **Conclusions.** CE in Ph negative clone in imatinib treated patients is not an infrequent finding. The significance of this type of CE is yet to be determined.

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NILOTINIB IN IMATINIB RESISTANT OR INTOLERANT PATIENTS WITH CHRONIC MYELOID LEUKEMIA - SINGLE CENTRE EXPERIENCE

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Backround. In the last decade we can see great success of targeted molecular therapy of chronic myeloid leukemia. The greatest benefit is basically in better tolerance of signal transduction inhibitors in comparison with conventional chemotherapy. The identification of imatinib caused a revolution in the CML treatment. However a group of patients that does not respond to imatinib or loose initially achieved response still alarms. The second generation of targeted molecular therapy represented by nilotinib prooved its position in treatment of patients with imatinib failure or intolerance as well as in the front line therapy in newly diagnosed chronic phase CML patients. **Aims.** Analyse second line nilotinib treatment in imatinib resistant or intolerant patients. **Methods.** In the period ranging from January 2007 to December 2011 there were twenty-one patients (13 men) treated with second line nilotinib, due to imatinib resistance (18 patients) or intolerance (3 patients) at the Clinic of Hematology and Blood Transfusion in Bratislava, Slovakia. The age of patients was between 23-77 years (median 49). According to Socal risk score, ten of them were classified in low, five in median and six in high risk score. Twenty patients were in chronic phase, one was in accelerated phase. All patients were pre-treated by imatinib from 1 to 74 months (median 27). Eighteen imatinib resistant patients were treated from 9 to 74 months (median 32,5). Four of them achieved transient CMR, 5 CCR and 9 PCgR. Three imatinib intolerant patients were treated from 1 to 17 months (median 3). **Results.** Patients were treated with nilo-

tinib 400mg bid 3-59 (median 22) months. One patient, who started treatment in accelerated phase, didn't achieve hematologic response and died after 6 months in blastic phase. One patient progressed to blastic phase after 24 months of nilotinib treatment, with transient CCR. Both patients were in Sokal low risk at the time of diagnosis. Regarding cytogenetic response, after 3 months, five patients from twentyone (23,8%) achieved MMR or CMR, six (28,6%) CCR and ten (47,6%) partial or none cytogenetic response. After 12 months, from 12 patients proceeding nilotinib treatment, 4 (33,3%) achieved CMR, 3 (25%) CCR and 5 (41,7%) PCgR. After 48 months, 1 patient is in CMR, 2 in CCR and 1 in PCgR. From 21 patients, 14 (66,7%) continue on nilotinib, 11-59 (median 33,5) months. 2 patients died and 5 changed to dasatinib because of resistance or intolerance to nilotinib. Adverse events occured by 12 patients (57,1%): 3 had hematologic, 4 nonhematologic and 5 combined toxicity. Hematologic toxicity was present by anemia (7 patients), thrombocytopenia (6 patients) and leukopenia (2 pts). Due to anemia complications 5 patients received erythropoetin, in 2 of them also hemotherapy was needed, with transient dose reduction. Nonhematologic toxicity was present by hepatotoxicity (8 patients), skin rash (5 patients), dyspnoe, acute pancreatitis and GIT intolerance. **Summary.** The second line treatment with nilotinib is an optimal option for patients resistant or intolerant to imatinib. However the regular monitoring is necessary because of possible adeverse effects. Our experience is similar to other centers.

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LONG TERM MOLECULAR RESPONSE IN IMATINIB TREATED CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. CML is characterized by t(9;22)(q34;q11) (Ph chromosome) and by expression of BCR-ABL fusion protein. Tyrosine kinase inhibition by imatinib opened a new era in the treatment of CML. In large number of CML patients complete cytogenetic response (CCgR) can be achieved with imatinib. European LeukemiaNet (ELN) recommendations made efforts in unifying follow up of patients throughout the Europe and recommended several scenarios concerning cytogenetic and molecular evaluation by real time reverse transcriptase PCR (RQ-PCR) as method of choice. In several countries, like Serbia, regular PCR follow up is not always available, and current national recommendations based on ELN proposals include regular cytogenetic and occasional molecular follow up. **Aims.** After establishment of molecular monitoring in Clinic of Hematology, Clinical Center of Serbia, based on "baseline"principle (EUTOS IS evaluation is pending), we evaluated depth of molecular response in cohort of CML patients on long lasting imatinib therapy and long lasting CCgR. **Patients and Methods.** Between July 2011 and February 2012, 40 CML patients on imatinib therapy in CCgR in our center were analyzed by RQ-PCR. Cytogenetic analysis was performed on unstimulated bone marrow cells applying HG-banding technique. Molecular response was assessed by RQ-PCR according to EAC protocol with primers and probes for TaqMan technology on ABI 7500 Real Time PCR System in peripheral blood samples. For absolute quantification were used standards for fusion BCR-ABL gene and control ABL gene (Ipsogene) and ratio BCR-ABL/ABL was compared with baseline (BCR-ABL/ABL ratio in 30 de novo CML patients). **Results.** Our group comprise of 40 CML patients in chronic phase, age 49. 5 yrs (20-74y), treated by imatinib for at least 3 years (36-363 months). 38 patients are on 400 mg/day and 2 patients are on 800 mg/day imatinib. All of them had normal karyotype (20 normal metaphases) on last cytogenetic evaluation. RQ-PCR analysis revealed complete molecular response (MR4. 0) in 30 patients (75%), major molecular response (MR3. 0) in 6 patients (15%). In this group, two patients were escalated to 800mg/d imatinib in second year of treatment because of no optimal cytogenetic response according to current proposals (ELN and national). Due to escalation, they achieved MR4. 0. In 4 patients (10%) optimal molecular response was not present (>0. 1% BCR-ABL/ABL) and during further follow up, we confirmed loss of previous complete cytogenetic response in 3 of them. Unsatisfactory molecular response by RQ-PCR suggested earlier evaluation and change in treatment (dose escalation or nilotinib). **Conclusions.** In our cohort of long term treated patients, molecular monitoring revealed good molecular response as almost 90% of patients achieved at least MR3. 0 after >3 years of treatment. Introduction of molecular monitoring will help us in further changes in treatment policy and further modification of national guidelines adopting international standards for molcular evaluation. As we have shown, regular molecular monitoring of CML patients would be useful for early prediction of undesirable events like loss of response.

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TYROSINE KINASE INHIBITORS IN CHRONIC MYELOID LEUKEMIA. HIPOGAMMAGLOBULINEMIA, ANOMALOUS PLASMATIC CELLS AND CLONALITY

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Background. The tyrosine kinase inhibitors (ITK): imatinib, dasatinib and nilotinib, when blocking specific tirosín-kinasas of the immune answer, interfere the T (inhibition TCR) and B (inhibition CD19) immune response. Certain publications show some coincidence between the imatinib administration and the development of multiple myeloma (MM). **Aims.** To determine if the use of ITK in patient of CML alters plasmatic cells and immunoglobulins. **Methods.** MLC patients in treatment with ITK in december 2010. Eighteen patients (mean age: 57 years (25-82)) in treatment (12 Imatinib, 3 Dasatinib, 3 Nilotinib) for a mean of 62 months (4-114). We quantified immunoglobulins (Igs) and the clonality is valued by Immunofijación (IF) and serum free light chains (sFLC) by Freelite-Turbidimetry. Immunofenotype (flow cytometry) of plasmatic cells in bone marrow (MO), looking of: CD38, CD138, CD19, CD56 and CD117 was made. **Results.** Mean level of Igs: 1191,94 mg/dl (634-3066). Hypogammaglobulinemia: 11 patients; hipergammaglobulinemia: 1 patient; normal level: 6 patients. Of the 18 cases, 15 MO were useful for valuation: 13 show plasmatic cells with aberrant fenotipo (CPa): CD19 (-) and CD56(+). only 2 of these 13 cases are CD56 (-). All they were CD117 (-). Percentage average of CPa in MO regarding total celularidad: 0,15% (0,002-0,47). percentage average of CPa regarding normal CP: 48% (3-100). There is not difference among the rate of Igs for age, time of treatment or immunofenotype. Less immunoglobulins level is observed in patients with imatinib regarding dasatinib and nilotinib (means of Igs: 983 mg/dl, 1382 mg/dl and 1835 mg/dl, respectively, Kruskal Wallis, tendency: p = 0,06). Two of the patients with hypogammaglobulinemia (women, 64 and 69 years) showed clonality IgG kappa; 0,11% and 0,34% of CPa on total medullary, and 70% and 50% have more than enough plasmatic normal, respectively. **Conclusions.** 1º. We suggest that the treatment with ITK is related with hypogammaglobulinemia, with the presence of aberrant populations of plasmatic cells in MO and, in smaller measure, with the existence of monoclonality. 2º. Although the presence of monoclonal gammopathy (general population: 0,7-1,7%; up to 3% in > 70 years; in our study: 11%) it doesn't condition the immediate progression to MM, the patients treaties with ITK should be watched over by means of sFLC, IF and screening of CPa

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EVALUATION OF THE SAFETY OF IMATINIB MESYLATE IN PATIENTS WITH CHRONIC MEYLOID LEUKEMIA

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Background. Imatinib Mesylate, a Tyrosine Kinase inhibitor, is presently the drug of choice for Chronic myeloid leukemia (CML). During therapy, a few patients develop myelosuppression and present with cytopenias. Adverse side-effects of the drug include edema, nausea,vomiting, diarrhea, cramps and cutaneous reactions. Adverse hematologic side-effects include anemia, neutropenia,and thrombocytopenia. **Aims.** The aim of this study was to evaluate the safety of imatinib therapy in patients with CML. **Methods.** Between December 2007 and October 2010. 200 patients with Ph+ CML-CP were included in the study. Eligibility criteria included-age 15 years and older, Eastern Cooperative Oncology Group-(ECOG) performance status of 0 to 2, adequate hepatic and renal functions, no prior imatinib therapy, non pregnant patients. CML-CP was defined as less than 10% blasts and less than 20% basophils in the peripheral blood and bone marrow and a platelet count of more than 100 x 10^9/L, but < 1000x10^9/L . Therapy was initiated with imatinib 400 mg orally daily and patients were monitored carefully for any adverse effects. Complete hemogram and liver function tests were done once in two weeks during the first month and there after monthly. Toxicities encountered were graded as per the National Cancer Institute common toxicity criteria version 2. Both hematologic and non-hematologic toxicities were managed with short interruptions of treatment and supportive measures, but the daily dose of imatinib was not reduced below 300 mg/ day. **Results.** Out of 200 cases included of CML in the study, all the cases were in chronic phase, male: female ratio was 0. 7:1 with median age 39. 06±13. 21(ranged from 15-81). The study showed that the commonest hematological side effects were grade 2 anemia 12. 5%, followed by leucopenia 8%, and thrombocytopenia 4%. While the most com-

mon non-hematological adverse effects were superficial edema and weight gain 51. 5%, followed by musculoskeletal pain 35. 5%, then gastro-intestinal(vomitting,diarrhea)19%. Fluid retention was the commonest side effect, which responded to low dose diuretics. Skin reactions were managed with corticosteroids and interruption of treatment, except in one case where the drug had to be discontinued permanently. Musculoskeletal pain responded to analgesics. The drug was safe and well tolerated. There were no deaths due to toxicity. **Concluions.** Imatinib mesylate is a well tolerated drug, and all undesirable effects could be ameliorated easily. The most common hematological side effect was anemia. while the non hematological side effects was fluid retention

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DELETION OF IKAROS GENE IS CONSIDERED AS AN ACQUIRED LESION IN THE BLAST CRISIS PHASE OF CHILDHOOD CHRONIC MYELOID LEUKEMIA: A CASE REPORT

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Background. Structural rearrangements in the gene IKZF1 (Ikaros) are considered as a strong adverse risk factor in leukemia, whether alone or in conjunction with other adverse prognostic markers. The most common prognostic factor is thePhiladelphia chromosome (Ph), representing the main characteristic of chronic myeloid leukemia (CML) and of a subset of acute lymphoblastic leukemia (ALL). **Aims.** To present the case of a pediatric patient with BCR/ABL1 positive CML who was treated with imatinib mesylate and achieved remission, but transformed to lymphoid blast crisis (BC) within 205 days following the diagnosis of CML with detected deletion of Ikaros in the phase of blastic transformation. **Methods.** A previously healthy 13-year-old girl was admitted to our unit due to arthralgia and leukocytosis (WBC 145 x 10^9/L, HB 11,2g/dL, HCT 35%, PLTs 588 x 10^9/L). Physical examination revealed hepatomegaly, splenomegaly and lymphadenopathy. The diagnosis of CML in chronic phase (CP) was based on the peripheral blood findings, on the bone marrow biopsy and on the histopathologic findings in the bone marrow biopsy. The cytogenetic analysis demonstrated 46,XX,t(9;22)(q34;q11). The presence of the translocation was confirmed by quantitative RT-PCR. The patient was treated with imatinib (400mg/m^2/day) and achieved morphologic, cytogenetic and molecular remission. **Results.** On the 205th day, the blood test revealed WBC 100 x 10^9/L, HB 13,3g/dL, HCT 39,5%, PLTs 205 x 10^9/L and that 73% of peripheral blood white blood cells were peripheral blood blasts. From the findings of the bone marrow studies, an ALL B-cell diagnosis was considered. At this time, cytogenetic findings indicated 46,XX,der(3)t(1;3)(q12;p26),t(9;22) (q34;q11). FISH analysis confirmed t(9;22)(q34;q11) and quantitative RT-PCR confirmed BCR/ABL1 gene fusion. FISH analyses testing for ETV6/RUNX1, PBX1/TCF3 fusion genes and MLL rearrangement were negative, but three copies of PBX1 were detected. MLPA analysis of the bone marrow sample at the diagnosis of ALL revealed a deletion in the Ikaros gene including exons 4-8 and a deletion in the EBF1 gene in exon 1. No copy number variation was found in the bone marrow sample from the CML diagnosis. The patient received chemotherapy according to the ALL IC-BFM 2009 treatment protocol plus imatinib at 400mg/m²/day. The patient failed to achieve complete remission and the duration of survival from the onset of blast crisis was 75 days. **Summary and Conclusions.** Genetic abnormalities of Ikaros are strongly associated with a very poor outcome and a high rate of relapse in childhood leukemia, independent of Ph status. Deletion of Ikaros was not detected in CP-CML, but was identified as an acquired lesion in the BC-CML sample. This finding suggests that alterations in Ikaros directly contribute to the pathogenesis of BCR/ABL1 ALL, in a way that remains to be determined.

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DASATINIB ACHIEVED A CMR AND AN IMPORTANT IMPROVEMENT IN CHRONIC GVHD IN A RELAPSED CHRONIC MYELOID LEUKEMIA PATIENT AFTER HAPLOIDENTICAL HCT

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Background. Dasatinib is widely used in postrasplant CML patients, reaching and maintaining molecular response. Its role in GVHD is not clear. Dasatinib is a potent inhibitor of TK Lck, implicated in the cascade of TCR activation and acts as a potent immunomodulator inhibiting T-cell activation, inflammation, leukocyte recruitment and host or allograft tissue destruction, the three steps involved in chronic GVHD (cGVHD). It's proved that dasatinib is able to inhibit PDGF-R, c-Kit and TGF-B receptors, implicated in cGVHD. **Aims.** To report outcomes with dasatinib therapy in a Ph+ CML patient who relapsed after haploidentical hematopoietic cell transplantation (HCT) with cGVHD. **Case Report**. A 40 years old man was admitted to our department in June 2009 with leukocytosis (WBC >103 x 10^9,blasts: 73%) and left leg deep vein thrombosis. Bone marrow aspiration (BMA) and immunophenotype revealed: ALL-B EGIL II suggestive of MPN; Cariotype: 23 X,Y; t(9;22); 9q-(100%). RT-PCR BCR/ABL Major: 67. 798% transcripts. He was diagnosed as Ph+ CML in Lymphoid Blast Crisis. He had normal physical examination, and no comorbidities. The patient received induction chemotherapy with PETHEMA ALL Ph+ 2002 regimen (vincristine, daunorubicin, Imatinib, prednisone and ITT), without response in day + 14 BMA. Intensification with IdAC, mitoxantrone was started, followed by two consolidation courses and submission to haploidentical HCT program. Complete remission was achieved. In September 2009 patient underwent haploidentical related donor HCT with ABO mismatch, and in day +100 post-HCT evaluation, he was in complete hematological and molecular response, complete chimerism and without Imatinib treatment. By March 2010 the patient was admitted to the hospital because of grade 3 hepatic cGVHD, skin and ophthalmic grade 2 cGVHD was also confirmed. Cyclosporine (CYA) immunosuppressive therapy after SCT was intensified with corticosteroids and mycophenolate mofetil (MFM); moderate improvement was achieved, liver enzymes were persistently elevated without symptoms. Patient remained in CCyR, CMR and complete chimerism. Immunosuppressive therapy with CYA and MFM was maintained with slow dose reduction and cGVHD was stable. By May 2011 Molecular relapse was confirmed and patient was admitted in our department. Immunosuppressive therapy was suspended gradually and the patient started treatment with Dasatinib 70mg/ day in May 30 2011. Mutational analysis was negative. No adverse effect was registered and it has not been discontinued because of toxicity; the dose was escalated to 70mg bid. CMV reactivation occurred on several times, starting preemptive treatment with valganciclovir each time and no CMV disease developed. In November 2011 the patient had MMR (BMA: BCR/ABL 0 transcripts in onetime), CCyR, CHR and complete chimerism. Moreover the patient has achieved cGVHD improvements, especially on skin affection, almost inappreciable at this time (Figure 1), liver enzymes decreased but have not normalized (360U/L to 220 U/L), and ocular involvement improved. It's remarkable that since molecular relapse, patient has been without immunosuppressive therapy. **Conclusions.** Dasatinib was well-tolerated and conduced to MMR, while achieving an improvement in liver dysfunction, skin lesions and ocular involvement of cGVHD. The role of dasatinib in cGVHD offers a window for investigation and future target therapy.

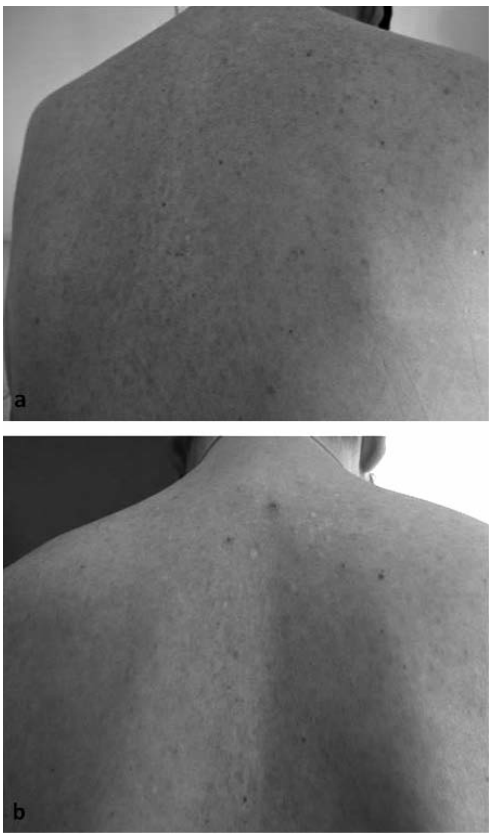

Figure 1.

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MGUS, MLUS, ICUS... AND NOW CML-US? REPORT OF TWO CASES

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Background. Detection of the Philadelphia (Ph) chromosome, a derivative of the reciprocal translocation t(9;22)(q34;q11. 2) is a recognized clinical hallmark for chronic myeloid leukemia (CML) diagnosis. CML is often suspected on the basis of pherypheral blood leukocytosis with "left shift", eosinophilia and basophilia. At the time of diagnosis, hematopoiesis results mostly from Ph+ clone, which is quickly debulked by target therapy with tyrosin kinase inhibitors (TKI). **Aims.** Here we report two interesting cases of patients incidentally diagnosed with CML, when cytogenetic analysis was performed without any clinical or laboratory data supporting an underlying Ph+ proliferative disorder. **Results.** A 65 years old woman was diagnosed with stage IVA Diffuse large B-cell lymphoma, from a biopsy of retroperitoneal mass in April 2011. Bone marrow staging was consistent with extended lymphoma infiltration (almost 70% of cellularity). Unexpectedly, bone marrow cytogenetic analysis showed 22% of 25 metaphases carrying the t(9;22) as sole alteration; RT-PCR identified b3a2 variant of BCR-ABL transcript. CBC count was normal, no splenomegaly was present. The patient underwent six courses of R-CHOP with achievement of complete remission confirmed by CT and PET scan. Bone marrow evaluation after the completion of chemotherapy did not show evidence of lymphoma or leukemia; cytogenetic was not evaluable but FISH analysis detected BCR-ABL fusion signal in 1,6% of 300 interphase nuclei; peripheral blood RT-PCR showed 1. 5% IS of BCR-ABL transcript . Normal CBC count, absence of splenomegaly and low disease burden prompted for careful molecular monitoring and TKI therapy was not commenced. The patient clinical status is currently good and there is no evidence of lymphoma recurrence. She is monitored every three months with RT-PCR analysis and the last evaluation of BCR-ABL transcript showed 5%IS. The second report concerns a 51-year-old man with type one diabetes mellitus who underwent kidney/pancreas transplantation in June 2005 and was subsequently treated with immunosuppressive therapy (prednisone, cyclosporine A, mycophenolate and tacrolimus). Shortly after transplant, he developed stable mild leukocytosis with neutrophilia, which was not furthly investigated as it was ascribed to steroid therapy. However, an unscheduled quantitative RT-PCR analysis performed in October 2011 revealed the presence of 1,01% IS BCR-ABL transcripts (both b3a2 and b2a2 variants). Bone marrow evaluation showed normal cellularity but cytogenetic s confirmed peripheral blood findings disclosing 12% of metaphases with t(9;22) translocation and 11%IS BCR-ABL transcript. As concomitant immunosuppression was considered a risk factor for disease progression, imatinib therapy was started but renal function worsened one month later. Creatinine clearance improved after TKI discontinuation. At present the patient is strictly monitored for TKIs resumption. **Summary.** These cases describe a peculiar presentation of CML (preclinical stage?) whose evolution is not clearly predictable and raise the issue of best treatment approach between watch and wait policy and TKI "pre-emptive" therapy. Molecular monitoring might help herapeutic decision making.

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MICROARRAY COMPARATIVE GENOMIC HYBRIDIZATION REVEALS ACCURATE CYTOGENETIC DIAGNOSIS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic Lymphocytic Leukemia (CLL) is characterized by the presence of malignant mature monoclonal B-cells (co-expressing CD5, CD19, and CD23) in peripheral blood, bone marrow and secondary lymphoid organs. CLL exhibits a highly variable clinical course with life expectancies ranging from a few years to many decades. Cytogenetic evaluation is of major importance in CLL and has revealed recurrent genomic alterations which have proven to be highly significant prognostic markers for this disease. In particular, patients with deletions of the short arm of chromosome 17 or the long arm of chromosome 11 show advanced disease, significantly shorter treatment free intervals, and shorter overall survival times. **Aims, Materials and Methods.** Current cytogenetic analysis in many diagnostic laboratories for patients with CLL involves routine FISH assays using targeted probes. DNA microarray technology (aCGH) supplies an alternative robust tool for large scale analysis of genomic imbalances in B-CLL. The aim of this study was to compare the aCGH results to the data obtained with FISH. For this purpose, peripheral blood (n=23) or bone marrow (n=3) from 26 previously untreated CLL patients were investigated on the Agilent 180k array (Agilent Technologies, AMADID 023363). Formerly, these cases were screened with a FISH probe panel for recurrent aberrations involving chromosomes 11q, 13q, 17p, and whole chromosome 12. Interphase FISH was performed according to standard procedures with commercially available probes (Abbott Molecular). **Results.** Array CGH screening identified in all but one patient the clonal imbalances recognised by FISH. Additional novel information was provided for 75% of the cases. This included gain of 2p, loss of 6q, gain of 8q, loss of 22q, loss of 14q, gain of 22. Analytical sensitivity for the detection of aberrant cells with aCGH was 10%. **Conclusions.** Our data demonstrate that aCGH and FISH results are highly comparable and support the implementation of aCGH into a clinical setting. Furthermore, the identification of additional (sub-microscopic) genomic imbalances will most likely lead to the identification of new prognostic markers important in the risk-stratification of CLL patients. An overview of the array CGH results will be presented.

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MOLECULAR KARYOTYPING IN DIAGNOSTICS OF HAEMATOLOGICAL MALIGNNACIES - HIGHLY INFORMATIVE AND COST NEUTRAL ALTERNATIVE TO CURRENT PRACTICES FOR DETECTION OF CLINICALLY RELEVANT GENOME IMBALANCES

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Background. Specific genome aberrations are recognised prognostic factors in haematological malignancies. FISH based genome risk stratification has been used in clinical decision-making for over a decade in particular for chronic progressive disorders such as chronic lymphocytic leukemia (CLL) and myeloid disorders (MDS and AML). Molecular karyotyping (array CGH) is gaining acceptance as an alternative that provides comprehensive whole genome scanning at high resolution. AIMTo assess the feasibility of molecular karyotyping for the routine diagnosis of haematological malignancies by comparing G banding, FISH and array CGH (aCGH). **Materials and Methods.** A total of 70 CLL and 50 MDS/AML samples were investigated. All had karyotyping and FISH tests done as part of the routine investigations. aCGH was performed using 8x60 oligonucleotide arrays (Agilent) with DNA from fresh p. blood/bone marrow samples and/or from fixed cells following manufacturer's protocols and bio-informatics routines (Z score and ADM1/2 algorithms). **Results.**The aCGH and G banding/FISH data were consistent for all presentation CLL and MDS/AML samples. Inconsistencies arose in 4 follow-up samples due to an aberrant cell population of less than 9%. Array data provide additional important novel information relevant to treatment and survival:In 40% of the 24 samples with 13q14 deletions detected by FISH, aCGH stratified the loss as class II/type 2 (>2Mb and the including RB1 gene), associated with high risk for disease progression. The total genome aberrations (TGA) at diagnosis varied between 3 and 17 with higher levels found in samples with p53 loss and/or high risk 13q14 deletions. Persistent disease was consistently associated with increased TGA count, while low TGA level coincided with clinical remission. In 21 out of 24 AML and 10 out of 16 MDS samples aCGH revealed novel, mostly multiple and recurrent hidden aberrations alone or in combination with other changes, indicating increased genome instability and hence offering a powerful novel clinical prognosticator. aCGH confirmed the preferential concomitant involvement of chromosomes 5 and 7 in MDS/AML as established by G banding and FISH but highlighted the role of cryptic losses as indicator for advanced disease irrespective of karyotypic features. Recurrent hidden loss of several gene regions, namely BUB1, TET2, TOP1, SYYK, TYK, NOTCH, ETV6 and RUNX1 were identified across all MDS/AML samples. **Conclusions.** aCGH is cost neutral when compared to currently used tests, so a strategy of array interrogation at presentation, followed by therapy response monitoring using FISH/molecular assays on a selected marker is a plausible alternative to routine practice. In cases where FISH has provided evidence for persistent disease, array screening on target cell isolates can assess genomic damage to assist risk stratification and treatment decisions. This preliminary study demonstrates that aCGH screening in routine settings offers a new powerful prognosticator for haematological malignancies

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INTERNATIONAL STANDARDIZATION OF QUANTITATIVE STUDIES OF BCR-ABL IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS IN RUSSIA

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Background. qPCR analysis of BCR-ABL is a tool for decision making in CML patients treated by tyrosine kinase inhibitors (TKI). According to European guidelines all qPCR studies in reference laboratories have to be internationally standardized. There are more than 100 clinical centers supervising more than 5000 CML patients in Russia, but only 15 Russian laboratories can provide accurate qPCR BCR-ABL analysis. So majority of centers need to collect samples in tubes with RNA-stabilizer and to send tubes to different laboratories for a long distance, and usually there are more than 24 hours from samples collection to analysis. Thus routine European protocols are not applicable to the majority of Russian laboratories. International standardization of BCR-ABL analysis taking into account specific Russian conditions is the vital problem. **Aims.** To create standardization protocol and perform standardization procedure in Russia in order to implement European CML monitoring guidelines and provide molecular monitoring according to International Scale (IS) for all Russian CML patients. **Methods.** Fifteen Russian molecular laboratories participated in standardization process. Informed consent was obtained when we used patients' blood samples. Flowchart of our project is shown on the scheme. Standardization process consisted of two main steps: local standardization (relating to Russian Reference laboratory) and international standardization (relating to ELN). Two protocols were standardized: RNA-unpreserved approach and method based on RNA stabilization.

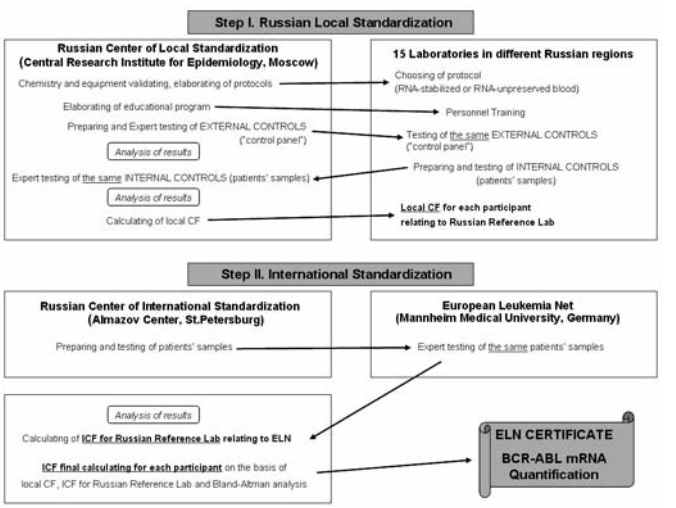

Figure 1. Flowchart of international standardization of quantitative studies of BCR-ABL in CML patients in Russia.

Results. 1. For the first time in the world quality assurance procedures for BCR-ABL molecular studies in the long-distance country are elaborated and implemented. 2. The reagents for both local BCR-ABL studies and for samples sent to distant laboratories are elaborated and certified. Reagents demonstrated high sensitivity (the majority of samples demonstrated 4. 0 log or 4. 5 log sensitivity). 3. Two universal protocols for processing of RNA-unpreserved and RNA-stabilized blood samples were approved. 4. Laboratory personnel were trained to work with reagents according protocols. 5. Twelve laboratories finalized standardization successfully and individual CFs were obtained. Individual iternational CFs (ICFs) ranged from 0. 48 to 1. 22 for the RNA-unpreserved protocol and from 0. 77 to 2. 05 for the RNA-stabilized samples. Three laboratories are still in process.

1350

IDENTIFICATION OF PROGNOSTIC RELEVANT GENETIC ABNORMALITIES IN MULTIPLE MYELOMA USING MICROARRAY-BASED GENOMIC PROFILING: EVALUATION OF DETECTION YIELD, SENSITIVITY AND COST-EFFICIENCY

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Background. Multiple myeloma (MM) is a heterogeneous disease with a highly variable clinical course. Genetic abnormalities such as t(4;14)(p16;q32), t(11;14)(q13;q32), t(14;16)(q32;q23), 17p loss, 13q loss, 1q gain, 1p loss and hyperdiploidy have been shown to provide prognostic information (Fonseca et al. Leukemia, 2009). The most common molecular-cytogenetic technique currently used to detect these genetic abnormalities is interphase fluorescence in situ hybridization (FISH) on purified plasma cells. **Aims.** Although interphase FISH is a well established approach, it is relatively laborious. We, therefore, we aimed to evaluate the sensitivity, cost-efficiency and detection yield of numerical genetic abnormalities using microarray-based genomic profiling. **Methods.** CD138+ cells (plasma cells) were enriched by an immuno-magnetic cell selection procedure (Stem Cell Technologies) from bone marrow samples of 13 MM patients. Microarray-based genomic profiling and data interpretation were performed as recently reported (Simons et al. Genes Chrom & Cancer, 2011). Interphase FISH on enriched plasma cells was performed using commercially available probes according to the manufacturer's specifications (Abbott Molecular for 13q-, 17p- and hyperdiploidy, and Kreatech Diagnostics for 1q gain and 1p loss). **Results.** All copy number alterations (CNAs) present in at least 20% of the cells, as determined by FISH, were also detected by microarray-based genomic profiling. In only one patient a minor sub-clone with loss of 17p, as detected by FISH in 13% of tetraploid cells was not detected by microarray analysis. The microarray-based genomic profiles of all 13 samples disclosed highly rearranged genomes, harboring 3 to 22 distinct CNAs or regions exhibiting acquired uniparental disomy (UPD), both recurrent as well as novel in nature. When microarray data analysis was focused on the loci also covered by the FISH panel (1p, 1q, 13q, 17p and hyperdiploidy), it appeared that microarray-based genomic profiling was cost effective. The use of microarray-based approaches may also have implications for the prognostification of MM by avoiding misinterpretation of tetraploid cells as exhibiting hyperdiploidy. The additional identification of local genomic instability (chromothripsis) that we observed in one patient, may serve as a novel high-risk factor. Summary and **Conclusions.** Our results indicate that microarray-based genomic profiling exhibits a high sensitivity and specificity in identifying MM-associated numerical abnormalities. Although balanced translocations cannot be identified this way, the detection CNAs is cost-effective. In addition, we show that microarray-based genomic profiling allows the detection of novel focal CNAs and acquired UPDs in patients. The prognostic significance of these novel CNAs and acquired UPDs have to be evaluated in prospective clinical trials.

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CYTOGENETICS OF CHILDHOOD AND ADOLESCENT ACUTE MYELOID LEUKEMIA (AML) IN GREECE

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Background. Diagnostic cytogenetics is recognized as one of the most significant prognostic factors in childhood and adolescent acute myeloid leukemia

(AML). **Aims.** We reviewed the clinical and cytogenetic characteristics of 140 AML patients ≤21 years of age at the time of diagnosis in order to investigate the cytogenetic features and their correlations with clinical characteristics and outcome in children and adolescents with AML in Greece. **Methods.** Cytogenetic analyses were performed on diagnostic unstimulated bone marrow cells, derived from children and adolescents with AML, between 1998 and 2010. Chromosome studies were carried out on trypsin G-banded chromosome preparations and karyotypes were described according to the ISCN 2009. **Results.** The sex ratio showed a male predominance (M/F:1. 33), especially in secondary AML (s-AML) patients (M/F:2. 4). According to FAB classification, M0 was found in 5. 88% of patients, M1 in 8. 4%, M2 in 30. 25%, M3 in 17. 65%, M4 in 14. 29%, M5 in 12. 61%, M6 in 5. 88% and M7 in 5. 04 %. Cytogenetic results were available in 124 patients (88. 6%). A normal karyotype was found in 23. 4% and an abnormal in 76. 6% of them. Chromosome analyses showed t(15;17) in 15. 32% of the available karyotypes, t(8;21) in 13. 7%, +8 in 10. 5%, 11q23 rearrangements and -7 in 8. 1% each, -X in 6. 5%, abn7p in 4. 8%, del(7q), del(9q) and abn12p in 4% each, del(6q), abn3q, inv(16) and -Y in 3. 2% each, t(9;11) and acquired +21 in 2. 4% each, t(11;19)(q23;p13. 1), t(16;21)(p11;q22), del(20q) and del(5q)/-5 in 1,6% each. Other abnormalities were identified in small numbers. Patients with t(15;17) showed the most favorable outcome with a 4-year OS of 93. 33%, followed by t(8;21) (72. 22%) while patients with 11q23 rearrangements and -7 showed the worst outcome with a 4-year OS of 38. 9% and 37. 5% respectively. Striking differences in genetic abnormalities and FAB subtypes were found between infants, children and adolescents. Infants <2 year old, showed the highest incidence of abnormal karyotypes (81. 25%) and complex karyotypes (53. 9%). The most common FAB subtypes were M5 and M7, and the most common abnormality was 11q23 rearrangement, followed by abnormalities of 7p. In children (>2 years until the age of 14 years), the most frequent abnormality was t(8;21), followed by -7 and the most common FAB subtype was M2, followed by M5. In adolescents (>14 years until the age of 21 years), the most frequent abnormality was t(15;17) followed by t(8;21) and trisomy 8 and the most common FAB subtype was M2 followed by M3 and M4. **Summary and Conclusions.** This study confirmed the high incidence of cytogenetic changes in children and adolescents. The frequencies of most chromosome aberrations and most FAB subtypes were comparable to previous studies. Interestingly, a high frequency of AML-M3 and a high frequency of t(15;17), +8 and -7 was observed in this cohort. Complex karyotypes did not emerge as an adverse prognostic feature in this study. The frequency of specific chromosome aberrations and FAB subtypes was found to vary between the AML age groups. Striking differences concerning mostly chromosome abnormalities and survival were also observed between de novo and s-AML.

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WITHDRAWN

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CHROMOSOME 1 ABNORMALITIES IN MULTIPLE MYELOMA

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Summary. Chromosome 1 is frequently affected in multiple myeloma (MM) and is related with tumor progression of the disease. The aim of our study was to describe the chromosome 1 rearrangements in MM. Cytogenetic examinations with G-banding techniques and fluorescent in situ hybridization (Fish) were performed on 56 untreated patients. Conventional cytogenetic study was successful in 46 cases and revealed 21 cases with clonal changes. Structural abnormalities of chromosome 1 were seen in 13 (62%) cases. All of them had complex karyotype. The most frequent aberrations of chromosome 1 were deletions of the short or long arm and unbalanced derivative translocations. Isochromosomes, isodicentrics or jumping translocations of chromosome 1 were also observed. More often the additional copy of chromosome 1 was rearrangement (8 cases). The following chromosomal bands were involved: 1p34, 1p32, 1p22, 1p13, 1p10, 1q10, 1q12 and 1q23. Fish examination of 25 patients with 1p36 and 1q21 specific DNA probes found clonal anomalies in 19 (76%) cases: 1p36 deletion in 6 cases, 1q21 deletion in 5 cases and 1q21 duplication in 9 cases. The pathological clones was always accompanied with minor clones carrying variety of abnormal Fish signal patterns. In conclusion, our results suggest that multiple loci in chromosome 1 are related with nonrandom chromosome aberrations. This confirms that possible hemizygosity of tumor suppressor genes or activation of oncogenes in chromosome 1 could lead to progression of MM. **Key Words.** multiple myeloma, chromosomal abnormality.

1354

DETECTION DNMT3A MUTATION IN ACUTE MYELOID LEUKEMIA BY PCR FROM CDNA AND SEQUENCING

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Background. DNMT3A mutation is one of the most common genetic mutations in AML patients, which related with poor clinical results; more than half of mutations located on R882 site. It's necessary to include detection of DNMT3A mutation into routine clinic diagnosis; however, the method should not be too expensive and complex. **Aims.** To establish a detection protocol simple and efficient for DNMT3A mutation in clinic. **Methods.** 1) Primers design: a pair of PCR and sequencing primers are designed from 2121-bp to 2782-bp of cDNA, (F: 5'-CAGTCCCTGCAATGACCTCT-3'; R: 5'-TTGTTTAACTTTGTGTCGCTACC-3'), which covering of most part of methyltransferase region (707aa-912aa), in the one PCR reaction production of 663-bp, including the highest mutation hotspots. 2) Patient samples: Bone marrow or peripheral blood samples were collected from 77 German patients in Department of Medicine A, Hematology and Oncology, University of Münster, Münster, Germany, the median age at diagnosis was 63 (range: 28-84) yrs; and 72 Chinese patients in Department of Hematology, Nanfang Hospital, Southern Medical University, China, the median age was 39 (range: 16-78) yrs, AML with PML-RARa and AML-ETO fusion gene were excluded. Samples collected after informed consent. (3) PCR and sequencing: The PCR reaction mixture consisted of 1ul sample cDNA, 250 nmol of both forward and reverse primers (Invitrogen), 200 umol dNTPs, 2. 5 ul 10×pfu PCR reaction buffer, 2 mmol MgCl2, 1 U Pfu DNA Polymerase (Fermentas), and sterile water up to the final volume of 25 uL. The first denaturation temperature was set at 94℃ for 5min, followed by 40 cycles of denaturation at 94℃ for 30s, annealing at 58℃ for 30s and extension at 72℃ for 90s, and ended with an extension at 72℃ for 7min. PCR production were then be sent to Invitrogen company (Shanghai, China) or by classical sequencing (using the Big Dye Terminator kit v. 3. 1). Each sample had at least 2 independent tests from both forward and reverse sequencing primers.

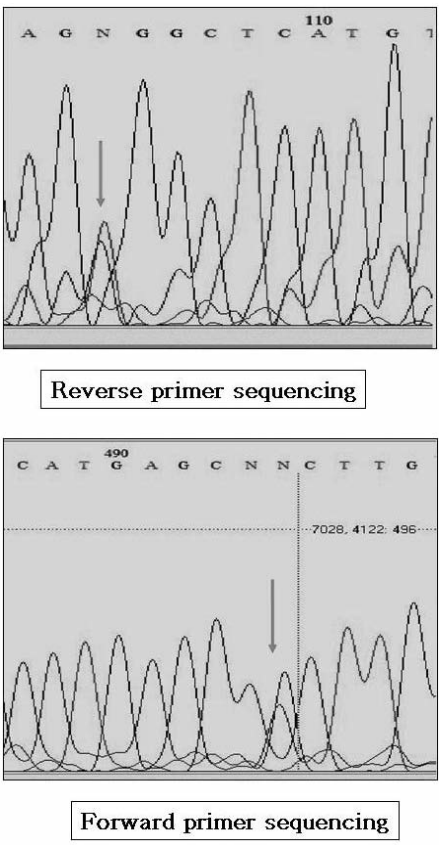

Figure 1. Double-peaks on R882 from one patient, with forward and reverse primers.

Results. 74 among 77 German samples have readable results, and 5 of them have potential DNMT3A R882 mutations (double-peaks on R882); while 69 among 72 Chinese patients have readable results, 2 of them have DNMT3A with R882C mutations and 5 have potential DNMT3A R882 mutations (double-peaks on R882). Double-peak single (Figure 1) at R882 position remained to be puzzled after sequencing repeated. Taken together, we have 12 positive or potential positive R882 mutations from 142 readable samples. **Summary and Conclusions.** We have detected DNMT3A mutations from both Chinese and German patients by our protocol, which can be use as a screening method at least, since the protocol is simple and economic. However, the puzzled results occurred in both Chinese and German, which may be improved by further detection with specific Taqman probe and primes, or new-generation sequencing.

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AMPLIFICATION OF 19P IS NONRANDOM EVENT IN ACUTE MEGAKARYOBLASTIC LEUKEMIA

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Acute megakaryoblastic leukemia (AML M7) is a very rare disease. Its exact genetic cause is under investigation, excluding t(1;22), which is found mostly in infants under 6 months. Hyperploid karyotype with complex aberrations is observed in the vast majority of cases. The most frequent abnormalities are 3q26 rearragements, -7, +8, +19 and +21. However the specific molecular markers in AML M7 are not found. Therefore our aim was to collect patients with successful karyotypes and search for recurrent cytogenetic abnormalities. We collected 11 cases of AML M7 (2009-2011). Cytogenetic analysis was performed on bone marrow cells, chromosomes were identified with G-banding technique. mFISH (Xcyte24, MetaSystems) and FISH (Vysis, Abbot Molecular) techniques were also used to evaluate chromosome aberrations. We had 7 patients with successful karyotypes. One patient had hypoploid karyotype with multiple rearrangements. One patient had Down syndrome and also exhibited complex hypoploid karyotype. Four patients had hyperploid karyotypes, three of them having additional chromosome 19. One patient had additional chromosome 3 as a sole abnormality. Using mFISH we elucidated karyotypes compared to those obtained by G-banding in all cases and identified the nature of marker chromosomes (see Table 1). We also had 4 patients without mithoses. Interphase FISH analysis was performed, but 3q26 rearragements, -7, +19 were not found in this group. In two patients amplification of 19p was found. In case 1 a marker chromosome was found in hyperploid karyotype. mFISH showed that it consists of the material of chromosome 19. Study with DNA probe LSI 19p13/19q13 (Vysis, Abbot Molecular) revealed that the derivative chromosome is composed of amplified 19p13 material. In case 5 mFISH showed hypoploid clone displaying jumping translocations involving chromosome 19 (t(2;5;19)(q12-14;q13;?), t(8;19)(q12;?), t(9;15;19)(q13;q11;?) etc). In this case we found that jumping material consists of amplified 19p13 region. So far, amplification of 19p is nonrandom event in AML M7 and usually correlates with complex karyotypes. The exact location and function of the amplified region in 19p is to be investigated, as well as clinical and biological aspects of this phenomenon.

Table 1.

Case	Age, months	Karyotype (G-banding)	Karyotype (mFISH+FISH)
1	18	42~44,XX,-1,-4,-6,-6,-8,-14,-15,-18,-19, +7mar,inc[cp20]	42~44,XX,+del(1)(q22),der(4)t(1;4)(?;q31),t(2;5)(q12-14;q13), der(6)(t(4;6)(?;?),-6,t(8;18)(q13;p11.2),t(8;19)(p12;p13), t(8;21)(q22;q11),t(9;15;19)(q13;q11;p13),-14,-18. ish t(8;19)amp(19p13)[cp4]/ 42,XX,+del(1)(q22),der(4)t(1;4)(?;q31),t(2;5;19)(q12-14;q13;p13), der(6)(t(4;6)(?;?),-6,t(8;18)(q13;p11.2),t(8;21)(q22;q11), t(9;15;19)(q13;q11;p13),-14,-18.ish t(2;5;19)amp(19p13)[1]/ 43,XX,+del(1)(q22),der(4)t(1;4)(?;q31),t(2;5)(q12-14;q13), der(6)(t(4;6)(?;?),-6,t(8;18)(q13;p11.2),t(8;19)(q12;p13), t(8;21)(q22;q11),t(9;15;19)(q13;q11;p13),-14,-18. ish t(8;19) amp(19p13)[1]
2	24	44,Y,-X,t(1;3)(q721;q?23-26),der(2),del(4)(q?), -7,t(7;9)?,-13,-15,-17,der(18),+21c,+2mar[9]/ 44,Y,-X,der(2),del(3)(q?),del(4)(q?), -7,-15,-17,der(18),+21c,+mar[17]	44,Y,t(X;4)(?;q28),t(X;9)(q21;p21),-7,der(13;15)(q21;dup13q?;p12), -17,t(17;18)(q12;q21),+21c[2]
3	11	51,XX,+2,+6 or +8,+10,+19,+21, +der(22)t(?4q31;22q13)[21]	51,XX,+2,t(4;14)(p11;q31),+6, t(7;22)(p15;q13),+10,+19,+21[3]
4	9	47,XY,-7,del(8)(q22),+19,+mar(r)[35]	47,XY,-7, +r(p22?p12q22?)del(8)(q22),+19[10]
5	57	47,XX,t(10;11)(p13;q21-23?), +mar(r?)[13]/46,XX[30]	47,XX,t(10;11)(p13;q21-23?),+der(19)(amp 19p13)[3]
6	12	50-51,XY,+6,+8,der(9),+19,+21,+mar[cp13]/ 46,XY[10]	50,XY,-4,+6,+8,t(9;11;15)(p13;q23;q14),+14,+19,+21[cp4]
7	12	47,XX,+3[8]/47,XX,+3,rear(3q21?-26?)[2]	47,XX,+3[2]

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EXPRESSION AND LOCALIZATION OF THE PRAME PROTEIN IN THE DIFFERENT CELL CULTURES

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Background. PRAME protein is one of the cancer-testis antigens common for haematological malignancies. There is no significant PRAME expression in blood and tissues of healthy donors. PRAME gene expression is reported for different types of leukaemia as well as for a number of solid tumours. PRAME mRNA evaluation is useful for MRD monitoring to prevent molecular relapse long before some other tests made it possible. PRAME protein cell localization has not been yet clear. **Aims.** To detect PRAME protein expression and cell localization in a number of cell cultures with different levels of PRAME mRNA expression. **Methods.** All cells after ethanol fixation were stained by anti-PRAME moAB's following by the secondary fluorescent AB treatment (Invitrogen) giving green signal. Cell nuclei were blue stained by Hoeshst. Green and blue in-cell staining was detected by Karl Zeiss Axiovert 40CL. Images were analysed by AxioVision software. Gene cloning and transfection were performed using standard techniques. **Results.** Earlier we developed 2 hybridoma lines (5D3F2-Prm and 6H8F12-Prm) secreting anti-PRAME moAB. In this study we used K562 erythroblastoid cell culture, human melanoma MEL-Kor, human fibroblasts WI-38 and mouse melanoma B16F10. In preliminary experiments by means of RQ-PCR we have shown that WI-38 and B16F10 were PRAME-negative whereas K562 and MEL-Kor were strongly PRAME-positive. We found that both 5D3F2-Prm and 6H8F12-Prm moAB's specifically bind PRAME protein. In case of highly PRAME expressing MEL-Kor and K562 it has been found that moAB's have different binding sites within the cells. It turned out that 5D3F2-Prm moAB (at dilution 1 to 100) binds mostly inside the cells, namely, to the nuclei, to the peri-nucleus area and within the cytoplasm. 6H8F12-Prm moAB not only stained the same cell compartments as 5D3F2-Prm, but also the cell surface (Figure 1 A,B). WI-38 and B16F10 that shown to not express PRAME mRNA was also negative at staining by 5D3F2-Prm and 6H8F12-Prm moAB's. On the contrary, those cell lines after transfection by PRAME-expressing pCEP4-PRAME plasmid became RQ-PCR PRAME positive and shown PRAME positivity following the staining by moAB's. In transfected cells both moAB's mostly bind nuclei creating green bright clusters (Figure 1 C,D), but distinct signal was detected also on the cell surface, especially when 6H8F12-Prm moAB was used. Interestingly, when both moAB's were used at less dilutions, fluorescent signal was detected only on the cell surface (Figure 1 G,H). It suggests that the AB's binding sites on the cell surface may obtain at these conditions some advantage to compare with intracellular binding sites. Not transfected WI-38 and B16F10 cells were never stained even at low dilutions of anti-PRAME moAB's (Figure 1 E,F),. When K562, Mel-Kor and WI-38 and B16F10 were stained alive, the fluorescent signal was observed only on the cell surface in the form of distinct green dots. **Conclusions.** Our data suggest that PRAME protein has at least two main cell localization sites revealed by moAB'S: nuclei and cell surface. The PRAME signals prone to form clusters both within the nucleus as well as on the cell surface. We believe that moAB's against PRAME may be useful for diagnostic purposes.

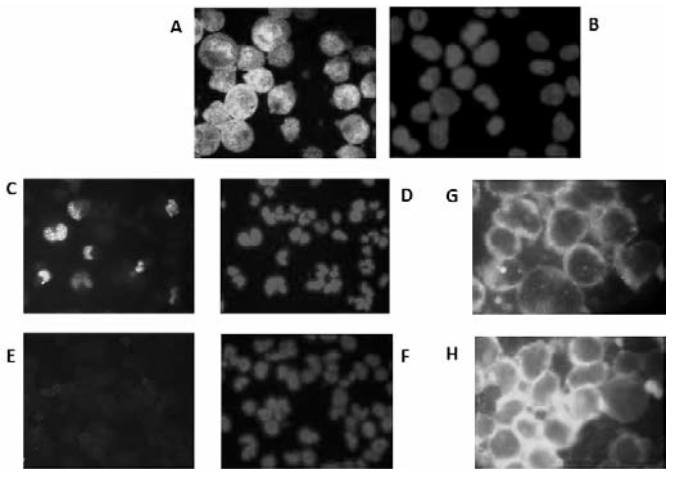

Figure 1.

1357

CONCURRENT REARRANGEMENTS OF BCL2, BCL3 AND BCL11A GENES RESULTING FROM THREE BALANCED TRANSLOCATIONS INVOLVING IMMUNOGLOBULIN GENES IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. Chromosome translocations including *IGH* rearrangement were thought to be relatively infrequent in chronic lymphocytic leukemia (CLL). This is not supported by more recent studies which have shown a higher incidence of *IGH@* rearrangements. B-CLL with t(14;19)(q32;q13) or t(2;14)(p16;q32) commonly presents with atypical cytological features, trisomy 12, and a progressive course of disease. Hence some have suggested it represents a distinct clinicopathological entity. This far three cases with concomitant translocations t(14;19) and t(2;14) have been described in literature. The t(18;22)(p11;q21), a rare variant of another *IGH* translocation t(14;18)(q32;q21), has been never found in combination with t(14;19) or t(2;14), although alone it has been most commonly observed in CLL. **Aims.** To present a case of B-CLL with three concomitant balanced translocations involving immunoglobulin genes in CLL. **Case Report.** We report a case of a 45 year -old female patient who was diagnosed with lymphoproliferative disease in 2004. The immunophenotype was typical for B-CLL, but because of the atypical morphologic features, four consecutive bone marrow examinations could not differentiate between CLL and mantle cell lymphoma. Initially negative FMC7 and Igl have become partially or completely positive in later analysis. CD38 has also gradually increased over years. The disease progressed in spite of aggressive treatment which started in 2007 (Fludarabine, Fludarabine Cyclophosphamide, Alemtuzumab). In 2011 the patient underwent an allogeneic hematopoietic stem cell transplantation and is currently in remission. Due to an aggressive course of disease and uncertain diagnosis, conventional cytogenetics was repeated in 2010. **Methods.** Conventional GTG banding was done after short term cultivation of bone marrow cells. Interphase FISH analysis was performed by Abbott DNA probes LSI IGH CCND1 and LSI IGH/BCL2. Translocations were confirmed by metaphase FISH analysis using Kreatech WCP probes. **Results.** In 2006 conventional cytogenetics confirmed trisomy 12 as a sole anomaly. Besides a normal female clone two leukemic clones were found in 2010. A small one with the isolated trisomy 12 and the main clone with three balanced translocations and trisomy 12: 47,XX,+12[2]/47,idem,t(2;14)(p13;q32),t(14;19)(q32;q13),t(18;22)(q21;q11)[16]/46,XX[2]. By interphase FISH analysis a biallelic rearrangement of *IGH@* and a rearrangement of *BCL2* (18q21) were observed. All three balanced translocations were also confirmed by metaphase FISH. **Conclusions.** We report here a case of CLL patient with three concomitant balanced translocations involving biallelic rearrangement of *IGH@* (14q32) and monoallelic rearrangement of *IGL@* (22q21). Partner genes involved are *BCL3, BCL11A,* and *BCL2* in t(14;19), t(2;14), and t(18;22) respectively. Although deregulation of *BCL3* and *BCL11A* were considered to play a major and primary role in the pathogenesis of CLL, in our case they were found as a result of a clonal evolution. Whereas atypical cytological features were observed already at the onset of disease (2004) translocations have evolved only after 2006 when trisomy 12 was found as the sole anomaly. Our case is additionally interesting since a rare t(18;22) was concurrently found with both *IGH@* translocations for the first time. Concurrent translocations are a further challenging topic in elucidation of clinical significance of *IGH@* rearrangements in CLL.

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COMPLEX THREE-WAY CHROMOSOMAL TRANSLOCATION T(3;3;5)(Q21;Q26. 2;Q32) - A NOVEL VARIANT OF ACUTE MYELOID LEUKAEMIA WITH INV(3)/T(3;3) WITH A BETTER PROGNOSIS?

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Background. AND AIM: Acute myeloid leukaemia (AML) with abnormalities of 3q represents 3. 5-4. 4% of all AML, but only that carrying inv(3)(q21q26. 2) or t(3;3)(q21;q26. 2) is recognized as a clinicopathological entity in the 2008 WHO classification. This type of AML shows typically an aggressive course and short survival. To date, other patients with AML carrying 3q abnormalities different to inv(3)/t(3;3) do not constitute a specific subgroup in this classification. We report a patient with a novel variant of AML with a t(3;3;5) (q21;q26. 2;q32), not previously described, with special attention to clinical and laboratory features. **Case Report.** A 62-year-old male without relevant antecedents attended to the emergency department referring dry cough, skin rash, fatigue and anorexia in the last two weeks. As remarkable data, the physical examination revealed a palpable hepatomegaly of 2-3 cm below the costal margin, and the analytical findings included pancytopenia (WBC 1. 3 x 10^9/L, neutrophils 0. 6 x 10^9/L, haemoglobin 68 g/L, MCV 108 fL, and platelet count 25 x 10^9/L). Peripheral blood smear examination revealed the presence of some isolated immature cell. Serology for hepatitis B, C, and HIV infection, and also autoimmunity screening, were negative, and a slight increase in serum lactic dehydrogenase (LDH) was observed. Bone marrow aspiration showed a very poor sample with a 7% of blasts, which were positive for CD34, CD13, CD38, CD61, CD36 and CD117. A marrow biopsy revealed hypercellularity with marked hyperplasia ("cobblestone"pattern) and dysplasia of megakaryocytic series, some "pockets"of blasts and red cell precursors clusters, and reticulin focal fibrosis (grade II). By immunohistochemistry, staining with CD31 showed a significant quantitative presence (>20%) of immature cells, also positive for CD34. Conventional cytogenetics evidenced the existence of a three-way translocation t(3;3;5)(q21;q26. 2;q32) in all metaphases analyzed, and *BCR-ABL1* was negative. Overexpressions of *EVI1, MN1* and *BAALC* were detected. The patient received intensive chemotherapy according to PETHEMA LAM/99 protocol with Idarubicin + Ara-C, but a marrow aspirate at +14 postinduction-day showed the persistence of blasts (5%). We decided to perform an allogeneic stem-cell transplant from a related donor (HLA-matched brother) in a reference center, which went off without significant adverse events. At present the patient has completed one year of post-transplant, always in complete remission and without complications. **Conclusions.** The disease in our patient shares some features with AML carrying inv(3) or t(3;3), mainly the histological appearance of marrow biopsy (increased small aberrant megakaryocytes, marrow fibrosis), but also the presence of hepatomegaly, an increased serum LDH and the overexpression of *EVI1*. However, the clinical course was much less aggressive. In a Spanish series of 35 patients with AML inv3/t(3;3) *(Raya et al,* EHA Congress 2011), median overall survival was only 7 months and median disease-free survival 3 months, even including those transplanted (29%). The evolution in our patient seems to be better, continuing in complete remission after 20 months from diagnosis.

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MOLECULAR CYTOGENETICS STUDY OF MULTIPLE MYELOMA ABOUT 30 TUNISIAN PATIENTS

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Background. Multiple myeloma (MM) is a malignant lymphoproliferative disease of B cells characterized by the accumulation of monoclonal plasma cells in bone marrow. Chromosomal abnormalities have played a major role in the disease progression of MM, among which we found by order of frequency: translocations involving 14q32 region (60%), especially t(11; 14)(q13, q32)(~20%), t(4; 14)(p16; q32)(~15%), t(14; 16)(q32; q23)(~5%), deletions of the long arm of chromosome 13(40-50%) and deletions of the short arm of chromosome 17(10%). **Aims.** We propose to study the chromosomal aberrations in 30 Tunisian patients with MM by conventional and molecular cytogenetic. **Methods.** The patients were prospectively analyzed by conventional karyotype, fluorescent in situ hybridization (FISH) and cIg-FISH which is a combination of cytoplasmic immunostaining of plasma cells and conventional FISH and compared the contribution of molecular cytogenetic techniques in the detection of chromosomal abnormalities. **Results.** Patients were firstly analyzed with conventional cytogenetic which detected four abnormal karyotypes (13. 4%). Molecular cytogenetic revealed at least one chromosomal abnormality in 16 patients (53. 4%), 14 of them didn't show any chromosomal abnormality by conventional karyotype. We detected by iFISH and cIg-FISH t(11;14) respectively in 23. 33% and 20%,t(4;14) in 3. 33% and 6. 67% and t(14;16) in 16, 67% and 20%. The same percentage of 6. 67% was found by the two techniques for deletion 13. We have not detected any 17p deletion. We also revealed association between t(14;16) and 13q deletion in 2 patients and a second association between t(4;14) and 13q deletion in 1 patient. The statistical study showed a sensitivity of 84. 6% with a confidence interval (CI_{95}%) of [53. 7% - 97. 3%] and a specificity of 82. 4% with a CI_{95}% equal to [55. 8% - 95. 3%] of cIg-FISH compared to iFISH. **Conclusions.** Our study indicates that cIg-FISH is the preferred method for the detection of chromosomal abnormalities involved in the MM. This will allow patients to be stratified into the new risk-adapted therapies based on cytogenetic and FISH.

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A CASE OF APLASTIC ANEMIA WITH KLINEFELTER'S SYNDROME

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Abstract. Aplastic anemia is characterized by diminished or absent precursors in the bone marrow. Klinefelter syndrome is the most common congenital abnormality causing primary hypogonadism. To our knowledge, the occurence of aplastic anemia with Klinefelter's syndrome has been reported twice before. We described a 22- year-old man who had suffered epistaxis and had been detected aplastic anemia and Klinefelter's syndrome at the time of searching of the etiology of pancytopenia. **Introduction.** Aplastic anemia is characterized by diminished or absent precursors in the bone marrow, in which normal hematopoetic cells are replaced by fat cells. In most patients, bone marrow hypoplasia is caused by immunological destruction of stemcells. The disease is estimated to occur in two to four subjects per million population per year (1,2). Klinefelter's syndrome is a variation of sex chromosome disorder characterized by hypogonadism, gynecomastia and azospermia and the most common congenital abnormality causing primary hypogonadism, occuring in approximately 1 in 1000 live male births(3,4). A case of Klinefelter's Syndrome with leukemia have been reported (7). **Case Report.** A 22-year-old man, suffered lasting epistaxis five hours and pancytopenia was detected, in his blood count white cells 3,2 x 10 ^{9}/L, hemoglobin 4,2 g/dL, hematocrit 12,1%, platelet 13 x 10 ^{9}/L. For the etiology of pancytopenia further tests were needed. Bone marrow aspirate revealed a severe hypocellular marrow, consisting of 1,6% blasts. Markers were negative for Hepatitis A, B, C, HIV and TORCH. Serological markers were also negative such as serum anti nuclear antibodies (ANA), double stranded deoxy ribonucleic acid (ds DNA). Chromosome analysis was performed with shortterm cultures of bone marrow cells. The karyotype was 47,XXY. In semen analysis azoospermia was detected. Gonadal hormone (normal range shown in brackets) such as testosterone 20. 22 pg/ml (8. 6-54. 6), follicle stimulating hormone 38. 88 u/l (1. 4-18. 1), luteinizing hormone 19. 6 u/l (1. 7-8. 6), prolactin 28. 17 ng/ml (2. 1-17. 7). With these laboratory findings we confirmed that his diagnoses were severe aplastic anemia and Klinefelter's Syndrome. After that,we performed patient and patient's family histocompatibility testing. A-38-year old brother was fully HLA-matched with him. So, we performed allogenic hematopoetic stem cell transplantation from his brother. Patient is still alive and we follow up him for two months after transplantation, recovery of bone marrow is achieved. **Conclusions.** Klinefelter's Syndrome patients have high risk of autoimmun disease. Aplastic anemia is also systemic autoimmune disease that hyperfunction of T cell causes to injury to hematopoetic tissue resulting in pancytopenia. Androgens stimulate hematopoietic system by various mechanisms. In the case reported androgen level was normal but FSH and LH levels were increased. So that can also increase levels of CD3+,CD4+ and CD8+, inducing bone failure. The mechanism of aplastic anemia and hyperprolactinemia in Klinefelter's syndrome was presumed to be elevated FSH and LH, but a detailed mechanism remains to be elucidated. And also we see that aplastic anemia accompanied by Klinefelter's syndrome dont influence the treatment. The patient has been responded to treatment like the other aplastic anemia patients.

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AN UNUSUAL CYTOGENETIC RESULT IN A PATIENT WITH ELLIPTOCYTOSIS, THROMBOCYTOPENIA AND A CONSTITUTIONAL MOSAIC 20Q DELETION

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We report on a 6-year old boy with a constitutional low-grade mosaic 20q11. 21q13. 32 deletion of 25. 6 Mb. He presented with a Robin anomaly, a short stature and a mild expressive speech delay. He was referred to us for cytogenetic examination of bone marrow because he also developed a mild anaemia, thrombocytopenia, elliptocytosis, a variable granulocytopenia and an increased HbF. Bone marrow examination showed no features of myelodysplasia, but nearly all bone marrow cells showed a 20q11q13 deletion; suggesting a relationship between the constitutional 20q deletion and the aberrant blood count. In several reported cases of myelodysplastic syndrome with marked elliptocytosis, an acquired del20q was observed. Congenital hereditary spherocytosis, irregular antibodies, folate and vitamin deficiency and growth hormone deficiency were excluded. To our knowledge this is the first patient with a constitutional 20q deletion of 25. 6 Mb and cytopenia with marked elliptocytosis and no myelodysplastic syndrome as yet.

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A MINOR BETA THALASSEMIA MASKED BY A NEW MUTANT OF THE HEMOGLOBIN A2

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Abstract. The elevation of hemoglobin A_2 (HbA_2) is an essential criterion in the diagnosis of minor β thalassemia. We report a case of minor β thalassemia with normal rate of HbA_2. **Case Report.** We report the case of ten years old boy, native of hafouz (governorate of Kairouan) which consults for an hypochromic and microcytic anemia refractory to iron treatment with a rate of Hb at 10 g/dl, a TCMH= 17,8 pg/GR, a VGM=59 fl and a normal rate of ferritin= 35 µg/l. The study of the hemoglobin revealed besides the HbA (93%), the HbA_2 (2,5%) and the HbF (1,5%) an additional minor abnormal fraction of Hb estimated to 3% migrating at middle distance between the HbA and the HbA_2. The phenotypic family's study revealed at the mother's one a minor β thalassemia isolated (the father was died) and we noted in the siblings (one brother and four sisters) tow anomalies of the hemoglobin (the β thalassemic line and the additional fraction of Hb) transmitted in an isolating or associating way. The genotypic study revealed the presence of a β^0 thalassemia mutation and showed that this minor abnormal fraction of Hb is a new mutant delta (δ) of the globin, which was called "Hb A_2 Pasteur"(δ59:E_3 lys→Asn (AAG→AAC). **Conclusions.** The presence of a mutant δ has reduced the rate of HbA_2 masking the β thalassemic line. The rigorous and methodic interpretation of the phenotypic data is primordial for not underestimate a minor β thalassemia whose diagnosis is decisive for genetic counselling.

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THE INVESTIGATION OF 621 PATIENTS SCREENED FOR THE JAK2 V617F MUTATION

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Background. Janus kinase (JAK) family of proteins are cytoplasmic protein kinases which are activating transcription factors by phosphorylation. During hematopoietic cell differentiation pathway, JAK2 plays an essential role by interacting with the hematopoietic growth factor signaling. It has been reported that somatic mutations which activate JAK2 kinase, could be responsible for erythrocytosis by inducing the erythrocytic proliferation. JAK2 exon 14 V617F mutation has been reported in most of patients with sporadic polycytemia vera. Studies has also shown that JAK2 V617F mutation may also be found in different types of leukemia. **Aims.** Here we aimed to analyze the patients screened for the JAK2 V617F mutation in our laboratory for molecular hematology . Between 2007-2012 years, the patients evaluated for the JAK2 V617F mutation were included the study. **Methods.** Mutation analysis was performed with the melting curve analysis and the allele specific PCR methods from the samples of bone marrow and/or peripheral blood. **Results.** A total 621 patient sample were analyzed for somatic JAK2 V617F mutation. For each year, about 40 % of the patients screened for JAK2 were found to have a somatic mutation. **Conclusions.** The allele specific PCR method has displayed a higher sensitivity and also allowed us to diagnose even very low persantage of mutant JAK2 in bone marrow / blood sample.

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TRANSLOCATION T(3;21)(Q26;Q22) IN PHILADELPHIA CHROMOSOME POSITIVE CHRONIC MYELOID LEUKEMIA PATIENT WITH BLAST CRISIS

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Background. The acquisition of secondary chromosomal aberrations in chronic myeloid leukemia (CML) patients with Philadelphia chromosome-positive karyotype signifies clonal evolution associated with disease progression to accelerated/blastic phase. Therefore, these aberrations are of clinical and biological importance. AimsWe report herein, a patient with Philadelphia chromosome-positive CML in whom a rare translocation t(3;21)(q26;q22),developed during disease progression. **Case Report.** A 35 year- aged man was diagnosed with CML. Chromosome analysis of bone marrow aspirate identified a Philadelphia translocation t(9; 22)(q34; q11). The patient received imatinib

treatment during 22 months. He was then lost to follow up. 12 months later, he developed blastic crisis, conventional cytogenetic analysis at that time revealed a reciprocal translocation t(3;21)(q26;q22) in the Philadelphia positive clone. He received chemotherapy with second tyrosine kinase inhibitor (dasatinib). Despite intensive care and supportive chemotherapy, the patient died of pulmonary infection and sepsis. **Discussion.** The t(3;21)(q26;q22) is a recurrent chromosomal abnormality in some cases of CML blast phase and in treatment-related myelodysplastic syndrome and acute myeloid leukemia. This translocation may result in the AML1-MDS1, AML1-Evi1 or AML1-EAP fusion transcripts. These secondary aberrations are thought to play a causative role in leukemic transformation of hematopoietic cells and should be the molecular basis of CML patient with t (3;21) (q26;q22) in blastic crisis. **Conclusions.** Our study indicates that the coexistence of BCR/ABL and t(3;21)(q26;q22) might play a pivotal role in the CML blast transformation and indicates a poor prognosis.

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APPROACHES TO GUIDE LUCATUMUMAB DOSE AND DOSING SCHEDULE BASED ON PHARMACOKINETIC (PK), PHARMACODYNAMIC (PD), RECEPTOR OCCUPANCY, AND TUMOR LOAD DATA FROM CYNOMOLGUS MONKEYS AND PATIENTS

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Background. Lucatumumab is a fully antagonistic human anti-CD40 monoclonal antibody that has demonstrated activity in preclinical and clinical studies and is hypothesized to act through dual mechanisms of inhibiting CD40L-CD40 signaling and inducing antibody-dependent cell-mediated cytotoxicity. Lucatumumab is being evaluated in several B-cell malignancies, including chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin lymphoma, and Hodgkin lymphoma. **Aims.** To guide dose selection and dosing frequency, PK and PD (measured by receptor occupancy) data from cynomolgus monkeys and patients and tumor volume data (measured by computed tomography and/or fluorodeoxyglucose positron emission tomography) from patients were analyzed using mixed-effect modeling. **Methods.** A NONMEM® dataset (ICON plc, Dublin, Ireland) was constructed with PK and PD data in 52 cynomolgus monkeys from 2 toxicology studies and 63 lymphoma patients from a phase 1a/2 open-label study. A target-mediated drug disposition (TMDD) model, which describes the nonlinear PK resulting from lucatumumab binding to CD40 on the cell surface, was adopted. Free lucatumumab concentrations in serum were described by a 2-compartment model. Population model-predicted target saturation profiles were used to estimate the percentage of patients that would maintain 90% target saturation over 18 weeks of lucatumumab treatment with various doses and dosing schedules. **Results.** Lucatumumab exhibited target-mediated disposition in cynomolgus monkeys and patients with chronic lymphocytic leukemia, multiple myeloma, and lymphoma, which was well described by the 2-compartment TMDD model. Earlier analysis demonstrated that the model-predicted target occupancy profiles closely mimic those of an independent ex vivo fluorescence-activated cell sorting-based lymphocyte occupancy assay in both cynomolgus monkeys and patients. The typical nonspecific linear clearance was 0. 251 L/day (coefficient of variation [CV]: 46%) and the nonlinear target-mediated clearance was 1. 18 L/day (CV: 170%) (clearance of target and complex [lymphoma patients]) and 0. 2 L/day (clearance of target and complex [cynomolgus monkeys]); the central volume of distribution was 2. 59 L (CV: 61%). The lucatumumab concentration required for 90% receptor occupancy was 1. 7 µg/mL. The projected percentage of patients (at a typical weight of 70 kg) who would maintain 90% target saturation at lucatumumab PK steady state (achieved after approximately week 7) is tabulated (Table 1). A consistent induction of soluble CD40 ligand upon dosing as well as an inverse relationship between free CD40 and PK concentration was observed, both of which support the receptor occupancy findings. **Summary and Conclusions.** The PK of lucatumumab exhibited target-mediated disposition, which was well described by the TMDD 2-compartment PK model. Lucatumumab administered on an every-2-weeks schedule provides the desired target receptor occupancy of ≥ 90% at a dose level of 4 to 6 mg/kg.

Table 1. Model-predicted percentage of patients showing 90% receptor occupancy.

	Lucatumumab Dose, %			
Lucatumumab schedule	1 mg/kg	3 mg/kg	4 mg/kg	6 mg/kg
q2wk	17.5	84.0	92.3	97.7
q3wk	2.5	53.0	68.3	85.8

q2wk, once every 2 weeks; q3wk, once every 3 weeks.

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CHARACTERIZATION OF THE INTERACTIONS BETWEEN MDR PROTEINS AND ANTICANCER AND ANTIINFLAMMATORY DRUGS

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Background. The multidrug resistance phenotype in hematological malignancies and other tumors can be associated with the overexpression of certain ABC transporters, termed MDR proteins. The P-glycoprotein -ABCB1/MDR1-mediated multidrug resistance was discovered first and probably still is the most widely observed mechanism in clinical multidrug resistance. Two other ABC transporters have been demonstrated to participate in the multidrug resistance: the multidrug resistance associated protein 1 (ABCC1/MRP1), and the breast cancer resistance protein (ABCG2/BCRP/MXR). Recently MDQ-kit™ was developed as an improved flow cytometric method to measure the functional activity of the three, clinically most relevant efflux transporters in the living cells. Following the successful multicenter performance evaluation of the kit, the SOLVO MDQ-kit has been approved for in vitro diagnostic use in the EU. The knowledge of the drug-transporter interactions is of pivotal importance because it may lead to treatment failures due to the efflux of chemotherapies from the target cells. **Aims**. The major goal of our study was to test several drugs used to treat diseases where the MDR phenomenon is common, such as chemotherapy of malignant diseases and immunosuppressive treatments in chronic inflammatory disorders. **Methods.** Drug - transporter interactions were characterized using relevant MDR transporter expressing in vitro test systems. The cytotoxicity assay is a widely used method to screen cytotoxic effects of the compounds. Cell lines overexpressing ABCB1/ABCC1/ABCG2 protein show resistance to drugs that are substrates of the given transporter. Calcein and Hoechst assay are useful whole cell based methods to detect interactions between the compound and the selected transporter. Molecules that are substrates or inhibitors of the transporter increase cellular fluorescence by inhibiting the ABC transporter mediated efflux of the dye. **Results.** We tested a panel of over 30 drugs (eg alkylating agents, nucleoside analogue, antimetabolites, etc). Overexpression of ABCB1/ABCC1/ABCG2 transporters resulted in increased resistance to 65%/52%/35% of compounds respectively. Some of these interactions have not been described previously. The inhibition of profile of the same set of drugs was tested using the same ABCB1/ABCC1/ABCG2 transporter expressing cell lines in Hoechst and Calcein assay. The correlation is limited between the two assays. Although, it should be taken into consideration that the cytotoxicity assay is a substrate assay and the dye efflux assay is an inhibition assay. **Conclusions.** The presented HT in vitro assays are well suited to predict interactions between molecules and MDR proteins. Passive permeability of the compound is a critical parameter. In general, high passive permeability compounds are good inhibitors. At the same time, high passive permeability may overcome resistance by MDR transporters. This may explain lack of correlations between the two assay types.

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ROLE OF CYTOKINES IN RESISTANCE TO ERYTHROPOIESIS STIMULATING AGENTS TREATMENT OF ANAEMIA IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS

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Background. Anaemia often develops in patients with lymphoproloferative disorders (LPD), decreases survival and worsens the quality of patients' life. Pathogenesis of the anaemia is based on the suppression of erythroid precursors by proinflammatory cytokines. Therefore erythropoiesis-stimulating agents (EPSA) are used as a pathogenetic therapy of anaemia in patients with LPD. However the resistance to EPSA develops in 30-40% of patients. **Aims.** To evaluate the influence of proinflammatory cytokines on the efficacy of anaemia correction with EPSA. **Methods.** We have examined the patients with multiple myeloma (n=10), chronic lymphocytic leukemia (n=5) and non-Hodgkin's lymphoma (n=6), age from 24 to 82 years (62. 7±12. 1). All patients have previously received not less than 3 cycles of programmed chemotherapy. Before the beginning of EPSA therapy we have evaluated the levels of proinflammatory cytokines: interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), interferon-γ (INF-γ); also a hemogram was controlled every two weeks during the whole treatment period. Anaemia with hemoglobin level less than 100 g/L was considered an indication to prescription of EPSA treatment. EPSA (Epoetin alpha) was administrated subcutaneously in dosage 150 IU/kg of body weight 3 times a week. The duration of EPSA treatment was 9. 0±3. 7 weeks on average (from 6 to 16 weeks). Patients with haemorrhages, severe hemol-

ysis, iron and B_{12} deficiency were not included in this study. Target level of Hb was 120 g/L. A positive response to treatment was considered in increasing of Hb concentration ≥20 g/L. **Results.** We have established that the initial level of proinflammatory cytokines in examined patients with anaemia was increased and it varied broadly: IL-6 - 301-810 pg/ml (487±142 pg/ml), IL-1β - 14-998 pg/ml (423±358 pg/ml), TNF-α - 12-156 pg/ml (57. 9±50. 3 pg/ml), and INF-γ - 47-1000 pg/ml (556±314 pg/ml). Nevertheless, there have not been observed any significant correlation between the cytokine level and the severity of anaemia (Hb concentration). However, there were positive correlations between levels of IL-1β and TNF-α (r=+0. 76; p<0. 05), IL-1β and INF-γ (r=+0. 48; p<0. 05), TNF-α and INF-γ (r=+0. 63; p<0. 05), evidencing their participance in suppression of erythroid precursors. There have not been observed any correlations between IL-6 and TNF-α, INF-γ, IL-1β (p>0. 05). Having compared the levels of proinflammatory cytokines and the efficacy of EPSA therapy we have shown negative correlation with IL-1β (r=-0. 59; p<0. 05), TNF-α (r=-0. 72; p<0. 05), INF-γ (r=-0. 50; p<0. 05). Thus, high level of cytokines decreases the probability of development of response to EPSA treatment. In the whole group the positive response was stated in 13 of 21 patients (61. 9%), i. e. Hb level increased from 89. 8±11. 7 g/L to 113. 1±19. 3 g/L (p<0. 002), red blood cell count - from 2. 98±0. 49×10^{12}/L to 3. 64±0. 88×10^{12}/L (p=0. 004), and Hct - from 27. 2±3. 0% to 35. 3±4. 3% (p<0. 001). **Conclusions.** Cytokines Il-1β, TNF-α, and INF-γ play a role in development of anaemia being suppressors of erythropoiesis and thus can serve as the predictors of response to EPSA therapy in patients with lymphoproliferative disorders.

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ROLE OF SERUM ERYTHROPOIETIN LEVEL AS A PREDICTOR OF RESPONSE TO ANAEMIA TREATMENT WITH RECOMBINANT HUMAN ERYTHROPOIETIN IN LYMPHOPROLIFERATIVE DISORDERS (LPD) PATIENTS

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Background. Anaemia in LPD patients often decreases the efficacy of antineoplastic therapy and survival of patients, also worsening their quality of life. Correction of anaemia is usually performed using RBC transfusions and administrated the Recombinant Human Erythropoietin (rHuEPO) which significantly reduce the need of transfusions and the risk of RBC transfusion-induced complications. Nevertheless, the efficacy of rHuEPO in LPD patients with anaemia is about 60-70%, while the cost this treatment is rather high. The use of prognostic factors predicting the efficacy of rHuEPO treatment will allow target administration of rHuEPO to patients who will probably respond to this therapy. **Aims.** To study the predictive value of level of serum erythropoietin (sEPO), measured in LPD patients with anaemia before the rHuEPO treatment. **Methods.** The studied group included patients with LPD (n=48): chronic lymphocytic leukemia (n=13), indolent non-Hodgkin's lymphoma and multiple myeloma (n=21). Patients' age varied from 24 to 82 years (62. 7±12. 1). rHuEPO were administrated in anaemia with Hb <10. 0 g/dL. All patients have previously received ≥3 cycles of programmed chemotherapy. 14 patients received RBC transfusions because of severe anaemia (Hb<8. 0 g/dL) before rHuEPO-therapy. We exncluded patients with haemorrhages, marked hemolysis, iron or B_{12} deficiency. rHuEPO were administrated at doses 150 IU/kg body weight 3 times a week subcutaneously. Before and during the rHuEPO treatment we controlled the haemogram. sEPO was evaluated before rHuEPO-therapy. The target Hb concentration was defined 12. 0 g/dL. Duration of treatment was withing 16 weeks. Positive response was defined as the increase of Hb ≥2. 0 g/dL. **Results.** Initial Hb level was found at 8. 30±1. 65 g/dL, erythrocytes - 2. 64±0. 64×10^{12}/L and hematocrit - 25. 9±5. 5%. Initial sEPO level in patients with anaemia varied broadly - from 3 to 1810 mIU/mL (in average 323±479 mIU/mL). Duration of rHuEPO-therapy was in average 9. 0±3. 7 weeks (from 4 to 16 weeks). In 30 of 48 patients (62. 5%; p<0. 05) positive response was stated. In the whole group of patients with positive response to rHuEPO treatment (n=30) there was observed a significant increase from baseline for the following parameters: Hb level - up to 12. 82±1. 03 g/dL (p<0. 01), erythrocytes - up to 3. 89±0. 67×10^{12}/L (p<0. 01) and Hct level - up to 38. 5±4. 2% (p<0. 01). Negative correlation between the initial sEPO level and efficacy of rHuEPO-therapy (r=-0. 36; n=48; p<0. 05) allowed us to conclude that a positive response was more commonly observed in patients with low sEPO at baseline. Having divided the patients to different groups depending on their baseline sEPO level we have revealed that in cases of initial sEPO level <130 mIU/mL (39±38 mIU/mL) positive response was observed in 20 of 25 patients (80%), with initial sEPo level 130-500 mIU/mL (265±107 mIU/mL) - in 7 of 11 (66. 7%), and with sEPO >500 mIU/mL (1016±561 mIU/mL) - in 3 of 12 (25%). In the former group of patients (with sEPO >500 mIU/mL) the efficacy of rHuEPO treatment was significantly lower than in first two groups (p<0. 05). **Summary.** Initial level of sEPO allows to predict the response to rHuEPO treatment and thus can be used in medical practice.

1369

IMPACT OF VKORC1 AND CYP2C9 POLYMORPHISMS ON INITIAL ANTICOAGULATION THERAPY WITH ACENOCOUMAROL

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Background. Although acenocoumarol has a narrow therapeutic range, it is the most prescribed drug in prevention and treatment of thromboembolism disorders inSpain. To reach the interindividual dose-requirement in the first weeks of anticoagulation is the main clinical goal, because of the high risk of bleeding or thrombosis during the first weeks. VKORC1 polymorphisms (rs9923231, rs9934438) are related to coumarin derivate sensitivity in the initial phase of therapy, and represent 20-25% of influence on interindividual dose-requirement. Pharmacogenetics can improve safety and effectiveness of acenocoumarol prior therapy has begun. **Aims.** Our goal was to evaluate the impact of CYP2C9, VKORC1, CYP2C19 and CYP4F2 variant genotypes on the required-dose, risk of overanticoagulation (bleeding) or underanticoagulation (thrombosis) and the time to achieve a therapeutic INR range. **Methods.** Hospitalized medical patients treated with acenocoumarol at the Virgen de las Nieves University Hospital (Granada, Spain) were genotyped by PCR Real-Time (CYP2C19*2 rs4244285), PCR-RFLP (VKORC1 rs9923231; CYP2C9*2 rs1799853 and CYP4F2 rs2108622) and direct sequencing (CYP2C9*3 rs1057910) and their clinical parameters were analyzed after starting anticoagulation therapy. All patients signed an informed consent. **Results.** Carriers AA for VKORC1 (rs9923231) were associated with a higher risk of INR>4 infirst days of anticoagulation. CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) were associated with delayed time to achieve therapeutic INR range. **Summary and Conclusions.** Patients carriers of described allelic variations for VKORC1, CYP2C9*2 and/or CYP2C9*3 needed lower doses at the beginning of anticoagulation than patients wild-type for these genes.

1370

DOES ANTI-PEG STATUS INFLUENCE THE EFFICACY OF PEG-CONJUGATED FILGRASTIM AS A TREATMENT?

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Background. PEG-conjugated Filgrastim is widely used as primary prophylaxis for febrile neutropenia in patients on myelosuppressive chemotherapy, however the effects of anti-PEG on its efficacy have not been adequately investigated. Recent published research indicates that as many as 25% of the normal population have detectable anti-PEG in their circulation with evidence to suggest that there is a direct correlation between those with anti-PEG and those with a reduced response rate to PEG-conjugated drug treatment. These significant findings indicate that anti-PEG status could influence the efficacy of PEG-conjugated drug treatments and needs further investigation in patients undergoing myelosuppressive treatment. **Aims.** To establish a laboratory protocol for the serological detection of anti-PEG in human plasma. Three cohorts of patient samples were aimed to be tested for the presence of detectable anti-PEG in their plasma; normal healthy subjects, haematology patients with no known exposure to PEG-conjugated Filgrastim and haematology patients with known exposure to PEG conjugated Filgrastim. The prevalence of the antibody in the normal healthy population and in the haematology patients not on PEG-conjugated drug treatments were determined to see if its occurrence is similar to that found in the general population by previous research carried out by Armstrong et al i.e. ~25%. The prevalence of anti-PEG in patients exposed to PEG-conjugated Filgrastim was also determined and together with their Full Blood Count results at the time of PEG-conjugated Filgrastim administration, a statistical assessment was made to determine whether or not there is any direct correlation between patients with anti-PEG and those with a reduced response rate to PEG-conjugated Filgrastim. **Methods.** A serological tube technique involving the preparation of PEGylated red cells was set up and this technique was then used to investigate whether any of the collected patient samples had anti-PEG in their circulation. The protocol used for anti-PEG detection was established by Armstrong et al. in 2003 and to date there is no published evidence of this novel detection method being used elsewhere. The set up of this serological technique for the purpose of this research study is the first of its kind inIreland. **Results.** There is a statistically significant difference in the proportion of plasma samples from the subjects with no known exposure to PEG conjugated Filgrastim which tested positive, i. e. 20%, and the proportion of sub-

jects with known exposure to PEG conjugated Filgrastim which tested positive, i. e. 0% (χ^2 = 5. 8, p = 0. 016). **Conclusions.** Anti bodies to PEG are less prevalent in haematology patients, likely due to the immuno-incompetence associated with their disease or the immune suppression associated with their chemotherapy. This suggests that PEG conjugated Filgrastim is likely to be a successful drug treatment for the reduction of febrile neutropenia in haematology patients.

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EVALUATION OF THE ROLE OF THE NEW INNOVANCE PFA P2Y TEST CARTRIDGE IN DETECTION OF CLOPIDOGREL RESISTANCE

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Background. Light transmittance aggregometry (LTA) has been extensively used in monitoring clopidogrel therapy. However, the availability of simple and rapid point-of-care platelet function assays is of great clinical importance. Thus, the manufacturer of the Platelet Function Analyzer (PFA)-100 System has recently provided the INNOVANCE PFA P2Y test cartridge. **Aims.** To assesss the capacity of the new test to detect reliably clopidogrel resistance. **Methods.** We enrolled 90 consecutive patients with coronary artery disease receiving chronic clopidogrel maintenance therapy in combination with aspirin. Twenty healthy volunteers served as controls. Clopidogrel resistance was simultaneously analyzed by the INNOVANCE PFA P2Y test cartridge, ADP-induced LTA, the flow-cytometric vasodilator-stimulated phosphoprotein (VASP)-phosphorylation assay and the multiple electrode aggregometry (Multiplate). In statistical calculations, for all platelet function assays we used both reference ranges as they are provided by the manufacturers and those derived from the 5th-95th percentiles of measurements in the group of healthy blood donors. Agreement among the 4 platelet function methods by two was assessed using Cohen's kappa coefficient. Spearman correlation coefficients were also used to test for associations between INNOVANCE PFA-100 P2Y CTs and ADP-induced LTA values. **Results.** According to the cut-off points for clopidogrel resistance proposed by the literature, agreement was fair between INNOVANCE PFA-100 P2Y and LTA (74. 4%) and Multiplate (75. 6%), while poor agreement was noticed with VASP assay (63. 3%). Based on cut-off points derived from the measurements from 20 healthy volunteers, agreement between the PFA-100 System and the other three methods improved compared to the previous cut-offs (76. 7%, 77. 8% and 67. 3%, respectively), but this improvement didn't reach statistical significance. There was also a statistically significant negative correlation of INNOVANCE PFA-100 P2Y CTs with peak (r=-0. 51, p<0. 001) and late aggregation (r=-0. 55, p<0. 001), Multiplate (r=-0. 47, p<0. 001) and VASP-assay values (r=-0. 41, p=0. 003) and a weak positive correlation with disaggregation values (r=0. 37, p=0. 003). **Conclusions.** The INNOVANCE PFA-100 P2Y seems to be comparable to other established platelet function assays in detecting clopidogrel resistance. However, the modest agreement among platelet function methods makes crucial the performance of platelet function testing with more than one technique in order to reliably identify poor responders to clopidogrel treatment.

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THE EXPRESSION OF RLIP76 AND MRP IN LEUKEMIA CELLS

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Background. RLIP76 is a membrane-located protein mediating the transport of multiple molecules, including both glutathione-conjugated and lipid-oxidized metabolites and drugs. Studies have shown that there is an increased expression of RLIP76 in several types of cancer cells, and that it plays a prominent role in drug resistance. While there are a large number of studies showing that leukemia cells can develop resistance to chemotherapeutic drugs due to the increased expression of multiple drug resistance (MDR) proteins, it isn't known if this resistance is also accompanied by a change in the expression of RLIP76. **Aims.** The aim of this study was to investigate RLIP76, MRP-1, and MRP-2 expression in leukemia cells. **Methods.** All of the investigations were performed at the mRNA level on the THP-1, REH, HEL, MV4-11, and U937 leukemia cell lines using quantitative PCR. **Results.** Each of the cell lines expressed comparable levels of RLIP76 and MRP-1, but the expression of MRP-2 was nearly 100 times lower than RLIP76 and MRP-1 in all cell lines. The REH cell line had the highest amount of RLIP76 and MRP-1 expression. While both the THP-1 and MV4-11 (AML M5) cell lines expressed more RLIP76 than MRP, the HEL (AML M6) cell line showed a greater amount of MRP expression. **Conclusions.** These results suggest that the RLIP76 gene could contribute to the development of chemotherapeutic drug resistance in leukemia cells.

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POLYMORPHISMS IN COAGULATION-RELATED GENES AND COLORECTAL CANCER PROGRESSION

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Background. We recently reported an association between factor V Leiden, prothrombin G20210A and factor XIII Val34Leu and colorectal cancer (CRC) onset. Our results did not support an effect on CRC risk for the other tested polymorphisms: PAI-1 4G/5G, *FGG* 10034C>T and *MTHFR* 677C>T. Earlier data from other studies did show a reduced survival for PAI-1 4G carriers with Dukes' stage A or B disease, and for *MTHFR* 677-T carriers with Dukes' stage C disease, but these data come from small studies. **Aims.** To determine the association between the above six variants and cancer progression (vital status, recurrences, three-year progression-free survival) in 1322 German colorectal cancer cases with at least three years of follow-up after diagnosis. **Methods.** Patients were included in the DACHS study (Darmkrebs: Chancen der Verhütung durch Screening) between 2003 and 2007 and were at least 30 years old with a first diagnosis of primary invasive, histologically confirmed CRC. About three years after diagnosis, follow-up information was gathered on local recurrences, secondary tumors and distant metastases by sending a questionnaire to patients' treating physicians. Information on vital status was ascertained through population registries. Mortality and recurrence risk during follow-up associated with gene variants was determined by calculating hazard ratios (HRs) and their 95% confidence intervals (CIs). Adjustments were made for age, sex, body mass index, disease stage, cancer detection circumstances and cancer site. In addition, progression-free survival within three years after diagnosis was compared across genotypes using logistic regression with adjustment for the above confounders. All participants gave written informed consent. **Results.** Of the 1322 patients, 328(25%) patients died during follow-up (251(19%) due to CRC) and 289(22%) patients developed a recurrence. The median follow-up time until recurrence, death or the end of follow-up, whichever occurred first, was 3. 0 years (range 0-7 years). In total 883 (67%) patients survived progression-free within three years after diagnosis. In contrast to earlier studies, we found a reduced CRC-related mortality for 177 patients with UICC stage II who were heterozygous for PAI-1 4G/5G (CRC-deaths 6%; HR 0. 35; 95% CI 0. 15-0. 83) compared to 73 5G/5G carriers (CRC-deaths 16%), but no clear reduction in CRC-related deaths for 121 4G/4G carriers (CRC-deaths 8%; HR 0. 60; 95% CI 0. 25-1. 43). We also found a reduced CRC-related mortality for 147 *FGG* 10034-CT carriers with UICC stage II (CRC-deaths 8%; HR 0. 48; 95% CI 0. 23-0. 98) and no CRC-related deaths among 28 TT carriers with stage II disease compared to 194 CC carriers (CRC-deaths 12%). Also in contrast to earlier studies, we found an improved progression-free survival for patients with colon cancer stage III for 111 *MTHFR* CT carriers (progression-free survival 69%; RR 2. 00; 95% 1. 10-3. 65) and 29 TT carriers (progression-free survival 79%; RR 3. 26; 95% 1. 18-8. 90) compared to the 96 CC carriers (progression-free survival 56%). **Summary and Conclusions.** We found a beneficial effect on CRC survival for patients carrying PAI-1 4G/5G, *FGG* 10034-T and *MTHFR* 677-T. Further studies are needed to validate our findings and to determine the prognostic value of these three variants.

1374

SHORT COURSE OF GRANULOCYTE-COLONY STIMULATING FACTOR TO ACCELERATE WOUND REPAIR IN PATIENTS UNDERGOING SURGERY FOR SACRO-COCCYGEAL PILONIDAL CYST: PROOF OF CONCEPT

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Background. Stem cells, namely the easily accessible bone marrow-derived cells (BMC) are reportedly capable of tissue repair in different damaged organs and might favour wound healing. The present study was undertaken to evaluate the feasibility and safety of BMC mobilization induced by granulocyte-colony stimulating factor (G-CSF) in patients undergoing surgery for sacro-coccygeal pilonidal cyst (SPC). To evaluate the possible clinical benefit of G-CSF in reducing time to complete resolution, a comparison with a control group receiving surgery without G-CSF was performed. **Methods.** Eight patients with complex SPC were included in this prospective trial. Patients were treated with G-CSF (5 µg/ kg b. i. d) for 3 consecutive days, standard surgical exeresis of the pilonidal cyst was scheduled on day 2 of mobilization. Mobilization was assessed in terms of circulating CD34+ cells and CFU-GM progenitors. **Results.** Mobilization of CD34+ cells and CFU-GM occurred in all patients, along with a marked increase in white blood cells (median peak value: 28,435/µL, day 3). G-CSF was well tolerated, no adverse events occurred. All patients received the planned surgical treatment without any complications. Interestingly, as detailed in Figure 1, G-CSF group patients had a median time to resolution (117 days, range 110-130) significantly shorter than controls (145 days, range 118-168) (p =0. 034). **Conclusions.** G-CSF administration, along with BMC mobilization, is feasible and well tolerated in patients undergoing surgery for SPC; clinical results compare favourably with those observed in controls not receiving G-CSF; the results suggest the potential use of G-CSF as additional treatment to accelerate wound healing in patients undergoing surgery.

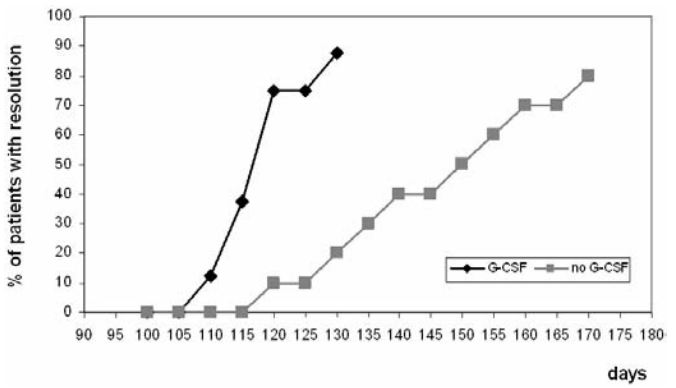

Figure 1.

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EFFICACY AND SAFETY OF LIPEGFILGRASTIM COMPARED WITH PEGFILGRASTIM IN PATIENTS WITH BREAST CANCER WHO ARE RECEIVING CHEMOTHERAPY

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Background. Cancer chemotherapy frequently causes neutropenia, leading to an increased risk of infections and delays in subsequent chemotherapy treatments. Pegfilgrastim is a pegylated recombinant form of granulocyte colony stimulating factor (G-CSF) that extends the half-life and requires less frequent dosing than nonpegylated G-CSF. Lipegfilgrastim is a glycosylated and pegylated G-CSF. **Aims.** The objective of this study was to compare the efficacy and safety of lipegfilgrastim and pegfilgrastim in chemotherapy-naïve patients with breast cancer who are candidates to receive docetaxel/doxorubicin. **Methods.** In this double-blind, randomized, active-controlled, noninferiority trial, patients with high-risk stage II, III, or IV breast cancer and an absolute neutrophil count ≥1. $5x10^9$ cells/L were randomly assigned to lipegfilgrastim 6 mg (n=101) or pegfilgrastim 6 mg (n=101). Study medication was injected subcutaneously on day 2 of the chemotherapy cycle (4 cycles maximum). Primary efficacy endpoint was the duration of severe neutropenia (days with an absolute neutropenia count <0. $5x10^9$ cells/L) during cycle 1. Secondary endpoints included the incidence of febrile neutropenia. The efficacy analysis population included patients who were randomized but did not have major protocol violations. **Results.** Overall, 37%, 46%, and 17% of patients had stage II, III, and IV breast cancer, respectively. The mean duration of severe neutropenia in cycle 1 was 0. 7 days in the lipegfilgrastim group and 0. 8 days in the pegfilgrastim group (poisson regression least squares mean [95% CI] -0. 218 [-0. 498 to 0. 062]). 56% and 49%, respectively, did not experience severe neutropenia in cycle 1. Three patients experienced febrile neutropenia; all were in the pegfilgrastim group during cycle 1. 28% of patients in the lipegfilgrastim group and 26% in the pegfilgrastim group had adverse events that the investigator considered to be related to study medication. Three and 7 patients, respectively had serious adverse events. **Conclusions.** The results of this study confirm that the efficacy of lipegfilgrastim is comparable with pegfilgrastim. No unexpected safety events were observed.

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ALTERATIONS IN LYMPHOCYTE SUBPOPULATIONS OF HEALTHY PRETERM AND TERM NEWBORNS

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Background. Relative, and absolute numbers of blood elements in lymphocyte groups, and subgroups change with life. Their numbers in preterms in various gestational weeks of their intrauterine life are expected be different when compared with newborns with mature immune systems. **Aims.** Health states of preterm, and term newborns can be evaluated using immunophenotyping performed based on the gestational week of the newborn. The aim of this research was determine major lymphocyte populations and subpopulations between premature and term newborns. **Methods.** The lymphocyte subsets from the cord blood of 122 premature and 44 term newborns were analyzed using two-color monoclonal antibodies and flow cytometry. Informed consent was obtained. The premature newborns were divided into three groups and the immunophenotypes of premature newborns with different age groups were compared. Here, the findings of a study of normal preterm and term subjects divided into cohorts of newborns, preterms (26-31 weeks, 32-35 weeks, 36-37 weeks), and terms (³38 weeks) are presented. **Results.** WBC counts decreased towards 32-35. gestational week. They increased a little bit, but at term they dropped to the levels below those detected at 26-31 weeks (p<0. 05). Relative WBC counts demonstrated a declining trend at a period close to term, while levels of $CD19^+$, $CD8^+$, $CD16^+$ 56^+ started to rise. Although absolute values changed during the interim period, values detected at 26-31 weeks, and term were close to each other. **Summary and Conclusions.** Numerical values related to immunity can be meaningful if compared between age-matched infants. Difference in relative rates of change can provide more information for the evaluaton of maturity.

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A CASE REPORT OF AN ACUTE DISTRESS RESPIRATORY SYNDROME FOLLOWING BY SEVERE PULMONARY EDEMA AFTER ADMINISTRATION OF G-CSF IN AUTOLOGOUS TRANSPLANTATION

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Introduction. Granulocyte-colony stimulating factor (G-CSF), a hematopoietic growth factor, is widely used to mobilization of peripheral blood stem cell

(PBSC), and to accelerate recovery from neutropenia after severe chemotherapy to decreasing the risk of infection. Adverse effects occur with G-CSF use in approximately 30% of cases, comprised predominantly of bone pain, headache, and general fatigue. Pulmonary toxicity is rare. Here we describe our first experience case of acute respiratory distress syndrome (ARDS) and pulmonary edema, following G-CSF administration. **Case Report.** We report the case of a patient who received G-CSF to accelerate recovery from neutropenia in ASCT for a refractory Hodgkin's disease, who had developed an ADRS followed by severe pulmonary edema. In April2008, a19-year-old female patient was diagnosed with nodular sclerosing Hodgkin's disease, Cervical and mediastinal lymphadenopathies were identified, and the patient was staged as unfavorable IIA. A complete remission obtained after six cycles of ABVD and 36gy initial focus sites complementary radiotherapy. In June 2009 patient relapse, with a ventro-basal pulmonary nodules infiltrates in computed tomography (CT) scan, a second complete remission was obtained after 2 cycles of DHAP. This patient was found to be in good health after physical examination, lung function testing, ECG as well as a chest x-ray; she was mobilized with G-CSF (lenolgrastim) 10 µg/kg for five days, and had received high dose chemotherapy with CBV regime included: cyclophosphamide, carmustine and etoposide, following by non-cryopreserved autologous stem cell transplantation. Beginning at day 6 lenograstim 263 µg/d was administered, and the WBC count had 0, 3 x109/L. After a second infusion of G-CSF, patient developed suddenly dyspnea, sinus tachycardia 135 bpm then 220 bpm, despite oxygen administration via mask9 litersper minute, arterial hypoxemia (Pao2/fraction of inspired oxygen [Fio2]:175 mmHg), bilateral infiltrates on a frontal chest radiograph, there was not heart failure, and no signs of pulmonary arterial embolism with contrast medium, that were indicative of ARDS. Four hour after, patient developed cough, retrosternal discomfort, and hemoptysis, with pulmonary hemorrhage in a plain radiograph of the chest. She was treated in reanimation with corticoid, furosemide, and amiodarone, and with empirical antibacterial (piperacilline and vancomycine) because of concomitant fever 38,5. After 3 days, there was marked improvement of the radiographic and physical findings, and she is alive at present, and remission has been maintained. **Discussion.** The lung injury is caused by neutrophil-dependent damage to the endothelial and epithelial barriers of the lung. Human granulocyte colony-stimulating factor (G-CSF) is the most important regulatory cytokine capable of stimulating neutrophil production and induces leaking of potentiation of proinflammatory cytokines from committed hematopoietic progenitor cells expression. **Conclusions.** A careful and close clinical monitoring of respiratory symptoms must be proposed in patients with antecedents of pulmonary disorders.

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EFFICACY AND SAFETY OF LIPEGFILGRASTIM IN PATIENTS WITH LUNG CANCER WHO ARE RECEIVING CHEMOTHERAPY

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Background. Cancer chemotherapy can cause neutropenia, leading to an increased risk of infections and delays in subsequent chemotherapy treatments. Recombinant granulocyte colony stimulating factors (G-CSFs) have been shown to promote the proliferation and differentiation of neutrophils in patients receiving chemotherapy. Lipegfilgrastim is a glycosylated and pegylated G-CSF. **Aims.** We evaluated the efficacy and safety of lipegfilgrastim in patients receiving cisplatin/etoposide for stage IIIb/IV non-small cell lung cancer. **Methods.** In this double-blind, randomized, placebo-controlled study, patients with ≥1. 5x10^9 neutrophils/L and ≥100x10^9 platelets/L were randomly assigned to lipegfilgrastim 6 mg (n=250) or placebo (n=125). Patients with high risk for febrile neutropenia were excluded. Study medication was injected subcutaneously on day 4 of the chemotherapy cycle (4 cycles maximum). The primary efficacy endpoint was the incidence of febrile neutropenia during cycle 1. Secondary endpoints included the duration of severe neutropenia (days with an absolute neutropenia count <0. 5x10^9 cells/L). **Results.** The population consisted of 87% men; the mean age was 58 years. Overall, 2. 4% of patients in the lipegfilgrastim group and 5. 6% of patients in the placebo group had febrile neutropenia during cycle 1 (odds ratio [95% CI]: 0. 390 [0. 121 to 1. 260], p=0. 1151). Mean duration of severe neutropenia in cycle 1 was 0. 6 and 2. 3 days, respectively (least squares mean difference [95% CI]: -1. 661 [-2. 089 to -1. 232], p<0. 0001). Thirty-two percent and 59%, respectively, experienced severe neutropenia in cycle 1 (odds ratio [95% CI]: 0. 325 [0. 206-0. 512], p <0. 0001). Fourteen percent and 10%, respectively, had adverse events that the investigator considered to be related to study medication. Twenty-three percent and 18%, respectively, had serious adverse events. **Conclusions.** The odds ratio for the incidence of febrile neutropenia in the lipegfilgrastim group is in line with published results for filgrastim and pegfilgrastim. The incidence and duration of severe neutropenia was significantly reduced in patients who received lipegfilgrastim. No unexpected safety events were observed.

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IMPACT OF NUTRITIONAL STATE OF INFANTS ON LYMPHOCYTE GROUPS AND SUBGROUPS

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Background. Advantages of breastfeeding with human milk containing immunologic components protective for the newborn are more prominent during the first 6 months of life. Extent of prophylaxis provided by breast milk *per se* is more comprehensive when compared with mixed feeding. Limited information is available on the effect of breastfeeding, and its duration on lymphocyte subgroups. **Aims.** In this study we wanted to reveal the potential effects of breastfeeding, and formula feeding within the first 6 months of life on immune system. **Methods.** Our study group consisted of 26-32-week-old 106 healthy infants who were breast (n=40) or formula-fed (n= 66). Formula-fed infants either received breast milk for the first 4 weeks their lives (n= 46) or only fed with milk substitutes (n=20). Informed consent was obtained. The lymphocyte subsets were analyzed using two-color monoclonal antibodies and flow cytometry. **Results.** Relative values of CD16 56, and CD8 increased in formula-fed infants, while relative values of other parametres declined. WBC, and absolute values of all other parametres were found to be higher in breast-fed infants. **Summary and Conclusions.** Breastfeeding, and its application for longer periods exert favourable effects on immune system.

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THE DIFFERENCE IN THE INCIDENCE AND THE TREND OF HEMATOLOGIC MALIGNANCIES IN JAPAN AND THE UNITED STATES.

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Background. Hematologic malignancies are heterogeneous with diverse etiology and prognosis. Therefore, evaluating the incidence and its trend according to subtype is very important. However, no detailed data from Asia is available. In addition, comprehensive comparison between Asian and Western is lacking. **Aims.** The aim of this study is to assess the incidence and its trend according to subtype of hematologic malignancy and to evaluate the difference between Japan and US. The diseases evaluated in this study were acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), multiple myeloma (MM), Hodgkin lymphoma (HL), and non-Hodgkin lymphoma (NHL) with detailed subtypes. **Methods.** We used the data from a population-based cancer registry in Japan and from the Surveillance Epidemiology and End Results (SEER) program 9. Registry data of Japan included 100,637 cases and the data of US, SEER 9 included 172,925 cases. The period covered in this analysis was 1993 to 2006 in Japan and 1993 to 2008 in the US. Rates of sex-specific, age-standardized incidence were estimated and the trend of disease is evaluated by joinpoint regression analysis. **Results.** The overall age-standardized incidence rate of all hematologic malignancies per 100,000 in 2006 was 14. 9 for males and 10. 0 for females in Japan, 33. 4 for males and 24. 3 for females in the US. The incidence is higher in the US than in Japan with most of subtypes especially for the lymphoid malignancies. The total numbers of hematologic malignancies are constantly increasing in both male and female in Japan and female in the US (Figure 1). As for details, male with AML in Japan, female with ALL in the US, both male and female with HL in Japan, male with NHL in the US and both male and female with NHL in Japanare constantly increasing. Interestingly, CML are constantly decreasing in this period in both Japan and the US. No obvious change in trend was seen with MM. Several lymphoma subtypes such as follicular lym-

phoma, mantle cell lymphoma, Burkitt lymphoma and the total numbers of T-cell lymphomas are constantly increasing while Hodgkin lymphoma-mixed cellularity is constantly decreasing in this period. **Summary and Conclusions.** We showed the difference in the incidence of hematologic malignancies between Japan and the US. As expected, incidence was higher in the US than in Japan with most disease. But the magnitude of difference varies between subtypes, such that Hodgkin lymphoma-nodular sclerosis and chronic lymphoid leukemia showed largest difference. Results of this study could be possible clues for future studies especially about etiology.

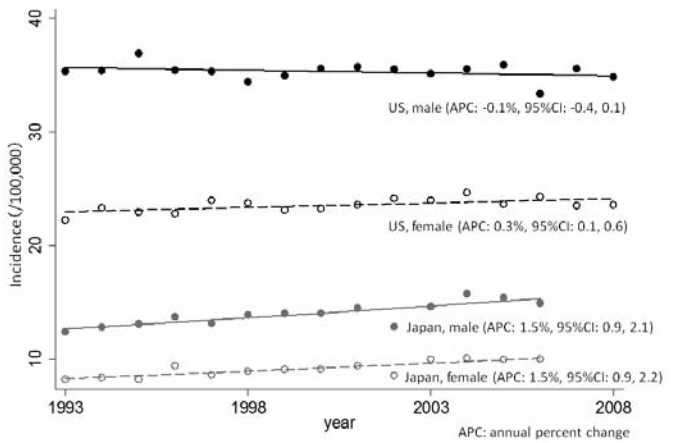

Figure 1.

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REDUCED CIRCULATING BLOOD LEVEL OF BDNF IS ASSOCIATED WITH CHEMOTHERAPY INDUCED PERIPHERAL NEUROPATHY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background. Chemotherapy Induced Peripheral Neuropathy (CIPN) is a major side effect and dose-limiting factor in cancer patients. Brain Derived Neurotrophic Factor (BDNF) is a neuronal growth factor that is responsible for the development, maintenance and repair of the peripheral nerve system. **Aims.** To identify alterations in the circulating blood levels of BDNF during CIPN development in patients with hematological malignancies. **Methods.** We used standard ELISA procedure to quantify the total level of BDNF in the blood plasma of patients with Multiple Myeloma (N=40), Hodgkin's (N=12) and Non-Hodgkin's lymphoma (N=25), prior to and during their treatment with the neurotoxic chemotherapies: Bortezomib, Vincristine and Vinblastine. CIPN development in these patients was assessed according to the cancer common toxicity criteria index. **Results.** We found that the plasma of patients who developed CIPN (SCORE≥2) during their treatment had lower BDNF level as compared to patients who did not develop CIPN (SCORE<2). (Mean±SEM in patients with CIPN vs. patients without CIPN 1. 71±0. 436ng/ml vs. 4. 62±0. 607ng/ml respectively P<0. 0001). No significant difference in the basal level of BDNF was observed between the different disease groups. **Conclusions.** These preliminary results indicate the involvement of loss of neuroprotective elements in the mechanism of CIPN and suggest the potential of blood circulating level of BDNF as a biomarker for CIPN development in patients with hematological malignancies.

1382

COST ANALYSIS OF SELF MANAGED VERSUS TRADITIONAL CARE ORAL ANTICOAGULATION THERAPY

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Background. Requirements for anticoagulation therapy are increasing due to an expansion of clinical indications for therapy. It has been shown that the evaluation of the oral anticoagulant therapy (OAT) effect can be achieved through frequent monitoring of international normalized ratio (INR). Portable coagulometer for patient self managed (PSM) of long term OAT is a fast, accurate, safe option for suitable trained patients of all ages. **Aims.** To evaluate the incremental cost between standard care and PSM for patients receiving long term OAT in the case of Chania district (Crete island). **Methods.** The current clinical practice is monitoring the INR and managing OAT by a physician. The alternative strategy is PSM where the measurement of INR is done by the patient using a portable equipment. Limitations of PSM procedure are that may be applicable to patients with ability to receive adequate training, capacity to perform the test, acceptable level of collaboration, compliance with the treatment, telephone contacts for advice and direct intervention to the anticoagulation clinic. **Results.** A cost minimization analysis was applied. All unit costs were estimated at 2011 prices in Greece and amortized over a 5-year period (Table 1). The cost evaluation was based on the costs of diagnostic procedures. Staff costs (doctor, scientist, technicians) were calculated on the basis of salary scale mid-points. Equipment costs were based on purchase prices. Patients in the traditional strategy would require 4 medical visits annually and those in PSM would have 1 visit. Patients having moderate hemorrhagic event were expected to stay 1-3 days in hospital. The cost of training was not included in the total cost. Furthermore both transportation costs of the patient and time lost from work were not calculated. It should be pointed out, according to the Greek national health system (GNHS),that the portable coagulometer is available free of charge for patients with atrial fibrillation, thrombophilia and mechanical heart valve whose the INR monitoring is inconvenient by public or private laboratory. **Conclusions.** PSM appears to be economically attractive over a 5-year period. This method requires identification and education of selected patients. Under strictly certain conditions, it is a funded option, in a number of patients in GNHS. The social benefit by improving the quality of life in the case of residents of isolated and rural areas seems to be significant.

Table 1. Estimated anticoagulation costs.

	Variable	*Unit cost(€)*	*Cost /5years(€)*
		Routine care	
Direct cost	INR analysis	4.05	243
	Medical visit	5.0	100
Indirect cost	Doctor salary/test	17.48	1048.8
	Technician & scientist salary/test	3.01	180.6
	Hospitalization 1-3 days (moderate complications)		514
Total cost			**2086.4**
		Patient self-	**managed**
Direct cost	Machine	678	678
	Test strip	5.08	610
	Medical visit	5	25
	Hospitalization 1-3days (moderate complications)		514
Total cost			**1827**

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A NEW SCORE TO DETERMINE THE PROBABILITY OF FINDING AN HLA IDENTICAL UNRELATED DONOR: A PROMISING EFFICIENT TIME AND COST SAVING METHOD

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Introduction. We recently demonstrated that a delayed time to find a compatible donor and also to proceed to allogeneic-HSCT can worsen the disease outcome (Michallet et al. ASH 2010). When a patient is presenting at our hospital for unrelated allogeneic-HSCT a donor search on the Bone Marrow Donor Worldwide (BMDW) registry is done, this query is limited to only 2-digits HLA typing of -A, -B, -DRB1 or -DQB1 loci. The identification of a compatible unrelated donor can be very long and expensive because of the limited HLA typing information in the registries. Tiercy et al. developed a computerized software that predicts the chance to find a suitable donor and the French transplant registry has recently developed a software called "Easy Match" that predicts the number of compatible donors for a given patient. **Aims.** To refine and acceler-

ate the process of donor search by combining the results of Tiercy and Easy-Match programs and define a new score for donor finding probability, in order to be time- and cost-efficient. **Materials and Methods.** We retrospectively analyzed 104 adult and 34 pediatric patients transplanted between 2009 and 2011 after finding an unrelated donor or cord blood units. Firstly, we analyzed the HLA characteristics of each patient as previously described by Tiercy et al. to provide a HLA score with Low, intermediate or high probability to identify a suitable identical donor. Then, we used the EasyMatch software which realize a "qualitative"analysis that consist on checking that each HLA recipient phenotype was found among all possible pair wise combinations of 2 haplotypes of the different sets of haplotypes. The EasyMatch software gives for each patient a number of potential donors sharing the same phenotype as the patient. **Results.** Our 138 patients were classified in 5 different categories (A to E) according to the combined results of the HLA score (Tiercy) and the Easy-Match software (Table 1). The results of the combination of the two methods allowed the definition of a new scoring system applicable to each patient and an economic strategy for an active search of donor. Score 0 (group A): 0% of chances to identify a 10/10 identical donor for the recipient. The choice of the source will be defined considering the HLA characteristics of the recipient; in case of class I rare allele or rare HLA-BC linkage disequilibrium; a cord blood unit will be easier and more rapidly available. A complementary help should be given by an associated analysis with 4-digit haplotypes as defined by Maiers (*Human Immunology 2007, 68; 779-788*). Score 1 (groups B and C): 50% of chances to identify a suitable 10/10 identical donor. Score 2 (groups D and E): from 75 to 100% of chances to identify a suitable 10/10 identical donor. **Conclusions.** The use of this new scoring system allows time and cost spare. In case of low chance to find a donor, physicians can have a fast redirection to find another treatment alternative in order to keep an optimal results.

Table 1.

	Tiercy Score	EasyMatch Number of potential donors	Patients	% of patients who found a donor and received allo-HSCT	
A	Low	0 or < 1	59	No HLA identical donor or cord blood unit : 100%	p=0,0003
B	Low	> 1	5	Non HLA identical donor or cord blood unit: 60%	p=ns
C	Intermediate	< or = 5	23	No HLA identical donor or cord blood unit: 56%	p=0,0008
D	Intermediate	> 5	16	HLA identical donor: 87%	p=ns
E	High	> 1	35	HLA identical donor: 100%	

1384

FAST INFUSION OF RITUXIMAB: RETROSPECTIVE SINGLE CENTER EXPERIENCE ACCORDING TO ROUTINE PRACTICE

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Background. Rituximab infusion according to the technical data sheet, generally requires 4 to 6 hours for de first infusion and 3-4 hrs for furthers. Several pilot studies have investigated the feasibility of faster infusion of Rituximab. We report a single center experience in fast infusion of Rituximab in second and subsequent administrations as routine practice. **Aims.** We analyze the security of patients receiving fast infusion of Rituximab and the rate of adverse events. **Methods.** We have retrospectively recorded data from patients treated with a fast rate infusion of Rituximab. To receive fast infusion, patients had to be treated with a first Rituximab infusion at an initial rate of 50 mg/hr, and in the absence of toxicity progressively increase by 50 mg/hr to a maximum of 400 mg/hr. Patients with infusion-related adverse effects were not suitable for fast infusion, as well as patients with significant cardiac disease. Patients received acetaminophen, dexchlorpheniramine and metylprednisolone before each rituximab infusion. In the absence of adverse event at maximum rate infusion, subsequent Rituximab cycles were administered at an initial rate of 400 mg/h. **Results.** Between 2006 and 2010, 882 rapid infusions were recorded in 102 patients with the following diagnostics: Folicular Lymphoma (49%), Diffuse Large Cell Lymphoma (18'6%), Chronic Lymphocytic Leukemia (15'7%), Marginal Lymphoma (11'8%), Lymphoplasmacytic Lymphoma (3'9%), Mantle Cell Lymphoma (11'8%) and Idiopathic Thrombocytopenic Purpura (1'2%). Administration of Rituximab was part of the following quimiotherapeutic regimens: R-CHOP in 395 infusions (44'78%), R-CNOP in 19 (2'15%), R-COP in 81 (9'18%), R-ESHAP in 16 (1'81%), R-FC in 6 (0'68%), R-MINE in 22 (2'49%), and R-Cisplatin in 6 (0'68%). Rituximab was administered as single agent in 286 infusions (32'43%). Among 882 rapid infusions, there were 2 adverse effect events (0'23%), consisting in hypotension and nausea and vomiting. The median infusion duration was 97'5 minutes (range 57-150 minutes). **Conclusions.** In our experience, fast infusion of Rituximab is a safe procedure that does not increase the rate of adverse events and results in a shorter hospitalization time, decreasing hospital's medical care load.

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NEW HAEMATOLOGY OUTREACH SERVICE PROVIDING TREATMENT AND SUPPORT FOR PATIENTS IN THE COMMUNITY: LOOKING AT FIRST 3 MONTHS OF DATA TO DETERMINE IF THE SERVICE CAN BE COST EFFECTIVE

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Background. The service was set up to provide a range of treatments and support for patients with haematological malignancies in the community. The service consists of two- haematology clinical nurses specialists funded by the Kay Kendall leukaemia foundation and offers a range of services in the patient's home such as; chemotherapy, IV infusions, platelet infusions, post high dose chemotherapy reviews and blood testing. All patients being treated within the service are under the care of University Hospital Birmingham. **Aims.** To reduce the number of hospital visits made by patients and where possible delivering treatment in the patients home. To provide support and information to patients undergoing treatment for haematological malignancies, identifying and managing complications and side effects of treatment as early as possible, whilst improving quality of life and the services for patients and being cost effective. **Methods.** 263 domiciliary visits made from December 2011 to February 2012. 90-chemotherapy deliveries. 8 platelet infusions. 14 intravenous infusions. 38 post high dose chemotherapy reviews and blood testing. 113 blood tests and x-matches for blood transfusions. Saving 67 return hospital transport journeys. 235 day unit visit. Prevention of 25 clinic visits. **Results.** Transport saving £6077, chemotherapy delivery saving £12000 Day unit time 130hours £25250 Clinic saving £3025. **Summary.** Our experience although in the early stages of a growing the service demonstrates that there are a number of savings to be made when delivering care and treatment in the community. Quality of life is also of huge importance when caring for haematology patient and the outreach service is not only saving on social costs for patients but is proving there are financial savings to be made on treatment delivery and time saved in hospital. Further audit and evaluation of the service is needed and expenses will need to be considered.

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MORTALITY MORBIDITY AND COST EFFECTIVENESS IN PATIENTS WITH FEBRILE NEUTROPENIA AN EXPERIENCE FROM QATAR

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Background. Febrile neutropenia is a major complication of chemotherapy, costly in terms of morbidity, mortality and associated financial expenditure. **Aims.** The present study was conducted with the goal of highlighting Febrile Neutropenia as a serious problem in Qatar, with the longer term objective of improved cancer survival, reduction in, morbidity, mortality length of stay (LOS) in hospital and costs in our existing scenario. **Patients and Methods.** A cross-sectional descriptive study was conducted on patients, ≥14 years, admitted with Febrile Neutropenia as a consequence of chemotherapy at Al-Amal Hospital in Qatar cancer centre from 1st January 2007 to 31st of December 2011. **Results.** A total of 175 patients [128 (73%) males and 47 (27%) females] were selected. The mean age was 41. 6 years; age range (14- 99) years. One hundred fifty three patients (87. 4 %) were ≤ 60 years, and twenty two patients (12. 6 %) were ≤ 60 years old. Almost 45% of the patients (n = 79) were previously healthy (no co-morbidity), hypertension was the most common co-morbidity (n = 35; 20%) followed by diabetes (n=26; 15%). Overall hospital mortality was 14%; (4. 5% for patients on granulocyte colony stimulating factor (G-CSF) pro-

phylaxis as against 9. 5% for those without). Febrile Neutropenia average medications cost is 5,330 USD/ patient and lengthen the hospital stay. The main cause of death was septic shock due to line related infections. Hematological malignancies, pneumonia and culture positivity were significantly associated with LOS and death. Those below 60 years of age were more than six times more likely to die than the patients above 60 years old, which is explained partially by predominance of younger population in Qatar (majority of Qatar populations whether native or expatriates are young). Bacteremia and line related infection conferred a 3-fold increase in the risk of death. **Conclusions.** The results of this study indicate that vital instability, culture positivity and hematological malignancies are high risk factors in chemotherapy induced Febrile Neutropenia. Identification of Febrile Neutropenia risk factors with poor outcomes may help in developing the protocols. This may help reduce the cost of cancer care as well as mortality and morbidity.

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MEASURE AND IMPACT OF NON-ADHERENCE TO BCR-ABL INHIBITORS FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA: OVERVIEW OF THE CURRENT LITERATURE

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Background. Recent studies have demonstrated that poor adherence to oral BCR-ABL inhibitors is an independent factor in the lack of success in treating patients with chronic myeloid leukemia (CML). **Aims.** The primary aim is to review the extent of non-adherence to BCR-ABL inhibitors and the resulting impact on clinical and economic outcomes. Secondary objectives include the evaluation of definitions and methods used to measure adherence and the identification of predictors of non-adherence to BCR-ABL inhibitors in patients with CML. **Methods.** A systematic literature review was completed using PubMed, Cochrane Library and EmBase, with no limits for time and language, including conference proceedings during the last three years (2009-2011). Search terms were related to CML, adherence/compliance, imatinib, dasatinib and nilotinib. The final studies included in the review reported adherence rates, adherence-related outcomes or proposed ways to improve adherence to BCR-ABL inhibitors. Because CML is a chronic disease, studies reporting persistence or discontinuation and subsequent reintroduction of medication were excluded from the review. **Results.** Out of a total of 342 studies, 38 met all the inclusion criteria and formed the basis of the literature review. Among the studies included in the review, adherence to BCR-ABL inhibitors in patients with CML varied from 25% to almost 100%. Additionally, multiple definitions and methods were used to measure adherence. Most of the studies (89%) examined the adherence to imatinib (first generation BCR-ABL inhibitor), with a few (8%) reporting on the adherence to dasatinib and nilotinib (second generation BCR-ABL inhibitors) only. The studies described that poor adherence can decrease event free survival, increase the risk of a suboptimal response, reduce the likelihood of achieving a complete cytogenetic response and molecular response and is associated with increased inpatient visits, length of stay in hospital, and healthcare costs. Adherence rates were found to be superior among patients receiving second generation BCR-ABL inhibitors compared to patients receiving imatinib. The number and severity of adverse events, duration of disease and duration of treatment, are key predictors for poor adherence. In addition, complexity of the treatment regimen has been identified as a significant factor increasing non-adherence in patients with CML. **Summary and Conclusions.** Adherence to BCR-ABL inhibitors is a critical component in ensuring long-term treatment efficacy. In addition, lower adherence has been associated with increased hospital resource utilization and healthcare costs. Among predictors for poor adherence to BCR-ABL inhibitors, adverse events and treatment regimen complexity require particular attention. Further research is needed to distinguish non-adherence across BCR-ABL inhibitors and to assess intervention initiatives that may enhance adherence to treatments for CML on a long-term basis.

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THE PRIMARY PRÉVENTION OF NEURAL TUBE DÉFECTS BY FOLIQUE ACID IN THE WILAYA OF BATNA

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Background. The terme of Neural tube défect(NTD) denotes a set of birth defects resulting from abnormal neural tube closure of variable size at the fourth week of embryonic developmentEtiology of NTD linked to food;famine of Holland 1944, where the incidence of spina bifida had doubled . Folate related NTD reported in 1965 by Hibbard . The preventive effect of folate evoked the 1960s, demonstrated the 1990s Folic acid supplementation is problematic because unplanned pregnanciesIt's a real public health problem and their impact is serious **Aims.** This work was undertaken to estimate the prevalence of folates deficiency in pregnant women in the wilaya of Batna. **Methods.** This is a semi longitudinal study which involved 375 parturients, mean age was 30. 11 ± 6. 9 years, attending antenatal clinics. **Results.** The prevalence of anemia was 59. 7% with 1. 34% of severe anemia, 33. 63% moderate and 65. 02% light. The prevalence of deficiency is, folate37. 6%, iron 49. 3% and vitamin B12 5. 3%. The prevalence of folates deficiency is 34. 9% in prégnant not anemic and 39. 5% in pregnant with anemia those 8. 5%, 14. 3% and 16. 6% respectively for first, second and third trimester of pregnancy. The prévalence of folates deficiency in pregnant women is even higher than the age of pregnancy is advanced. As for the assessment of knowledge of folic acid for pregnant revealed that 19. 7% had heard of folic acid, 8. 3% are able to cite at least one food rich in folate and 7. 7% of pregnant may clarify the rôle of periconceptional folic acid. Their source of information was midwives followed by the media. **Summary and Conclusions.** The prevalence of folate deficiency in pregnant women in this study is high preventive measures are essential, especially health education Donc, en termes de prévention de la carence en économie de la santé folique chez les femmes enceintes est plus intéressante que la gestion des conséquences de cette carence comprennent les cas de spina-bifida.

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RECONSTRUCTION OF HEMATOPOIETIC INDUCTIVE MICROENVIRONMENT AFTER TRANSPLANTATION OF VCAM-1-MODIFIED HUMAN UMBILICAL CORD BLOOD STROMAL CELLS

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The hematopoietic inductive microenvironment (HIM) is where hematopoietic stem/progenitor cells grow and develop. Hematopoietic stromal cells were the key components of the HIM. In our previous study, we had successfully cultured and isolated human cord blood-derived stromal cells (HUCBSCs) and demonstrated that they could secret hemopoietic growth factors such as GM-CSF, TPO, and SCF. However, it is still controversial whether HUCBSCs can be used for reconstruction of HIM. In this study, we first established a co-culture system of HUCBSCs and cord blood $CD34^+$ cells and then determined that using HUCBSCs as the adherent layer had significantly more newly formed colonies of each hematopoietic lineage than the control group, indicating that HUCBSCs had the ability to promote the proliferation of hematopoietic stem cells/progenitor cells. Furthermore, the number of colonies was significantly higher in vascular cell adhesion molecule-1 (VCAM-1)-modified HUCBSCs, suggesting that the ability of HUCBSCs in promoting the proliferation of hematopoietic stem cells/progenitor cells was further enhanced after having been modified with VCAM-1. Next, HUCBSCs were infused into a radiation-damaged animal model, in which the recovery of hematopoiesis was observed. The results demonstrate that the transplanted HUCBSCs were 'homed in' to bone marrow and played roles in promoting the recovery of irradiation-induced hematopoietic damage and repairing HIM. Compared with the control group, the HUCBSC group had significantly superior effectiveness in terms of the recovery time for hemogram and myelogram, CFU-F, CFU-GM, BFU-E, and CFU-Meg. Such differences were even more significant in VCAM-1-modified HUCBSCs group. We suggest that HUCBSCs are able to restore the functions of HIM and promote the recovery of radiation-induced hematopoietic damage. VCAM-1 plays an important role in supporting the repair of HIM damage.

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OXYGEN SENSING PATHWAY DEFECT DUE TO AN UNUSUAL COMPOUND HETEROZYGOTY FOR VHL GENES MUTATIONS: P. ASP126 (D126G) AND P. LEU188 VAL (L188V)

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Background. Oxygen sensing pathway defects, known as Chuvash Polycythemia, are rare autosomal recessive conditions. The von Hippel - Lindau gene (VHL gene) protein product is the main negative regulator of the hypoxia sensing pathway through its rapid clearance of hypoxia inducible factor-1 (HIF-1 alpha) under normoxic condition. Some VHL mutations will cause derangement of the oxygen sensing pathway with resulting persistent increased secretion of erythropoietin. This condition, in majority of reported cases,

responds well to regular venesection and often has a benign course even if the increased risk of thrombo-embolic events and some tumours such as pheochromocytoma and vertebral hemangioma have been reported. Few cases of Chuvash Polycythemia have been reported worldwide and none in Australia until this case. **Case Report.** A 7 year old boy, with no significant past medical history, was referred to the Royal Children's Hospital, Melbourne with 6 months of intermittent frontal headache and 3 days of lethargy and drowsiness following a mild head injury. The clinical findings and investigations were consistent with polycythemia (Hb 224g/L). Serum erythropoietin level was markedly raised. Extensive investigations included imaging of brain and abdomen/ pelvis, echocardiography, overnight oximetry, 2,3 DPG level and activity, Hb electrophoresis, chromosome analysis and Hb oxygen affinity. All returned within normal limits. Bone marrow biopsy revealed erythroid hyperplasia with mildly reduced granulopoiesis. VHL mutation testing confirmed the diagnosis of Chuvash Polycythemia due to compound heterozygoty: p. Asp126 (D126G) and p. Leu188 Val (L188V). To the best of our knowledge, although the two mutations identified have been previously reported in isolation, no case of similar combined heterozygoty have been reported in patients with Chuvash Polycythemia. Whether this explains his poor response to regular venesection, hydroxyurea and digoxin therapy, and unusual clinical course remains to be determined. Regular venesection was complicated by iron deficiency and pica. Hydroxyurea and Digoxin therapy were unsuccessful. **Conclusions.** VHL mutation testing should be part of the investigation of a patient with unexplained polycythemia. It is important to establish this diagnosis to facilitate the medical follow up, rationalise surveillance and avoid unnecessary ongoing investigations. These abnormalities are not confined to Russia despite few cases reported elsewhere.

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REMARKABLE PROMOTERS: ACCOUNTING FOR SEQUENCE VARIATION IN THE CONTEXT OF THE REGULATION OF GENE EXPRESSION IN HAEMATOPOIESIS

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Background. The DNA sequences of human promoters have a characteristic consensus sequence although variation about the consensus sequence has received little attention. In particular, we have found correlations between the bases at different positions amongst the sequences. **Aims.** We have sought to take account of the correlations using multivariate analysis to define groups of promoters with similar sequences. We have found a pattern amongst the eigenvalues, not previously described (we believe), and we examine which particular promoters have led to this pattern. **Methods.** The 60-base sequences of 1975 human promoters were extracted from the Eukaryotic Promoter Database. There were sequences for several factors involved in haematopoiesis and the differentiation of precursor cells, in particular, interleukins, CSF's, TNF-alpha and TGF-beta. Each base was coded by two binary digits and correlation coefficients were calculated between all pairs of the resulting 120 variables. A principal component analysis of the 120 variables was carried out and the eigenvectors were used to calculate principal components by which the promoters were classified. The principal components analysis was repeated using data which had been randomised to break down the correlations. **Results.** Notable correlations were found in the region of the transcription initiation site and the TATA site. After the principal component analysis of both the original and randomised data, the eigenvalues showed a downward trend. However, the first 5 eigenvalues were considerably larger for the original than the randomised data. The first eigenvalue was 0. 86 for the original data but only 0. 37 for the randomised, thus an excess variance of 0. 49 was observed. Certain promoters with a large first principal component (positive or negative) accounted for this excess variance and we term them *remarkable* promoters (positive: group 1a; negative: group 1b). Similarly, further remarkable promoters were identified for the next major principal components. Group 1a comprised 120 remarkable promoters, including TGFB1I1 (TGF-beta 1, induced transcript 1); group 1b, 202 promoters, including IL1A, IL1B, IL2, IL2RA 1 (Interleukin-2 receptor, IL2RA gene, alternative promoter 1), IL4, IL5, IL6 2; CSF2 (GM-CSF, gene) and TNFSF10 (TNF ligand superfamily, member 10). Group 2a comprised 90 promoters including CSF2RA (GM-CSF receptor alpha); group 2b, 139 promoters including TGFB1I1 (again). Group 3a contained 83 remarkable promotes and group 3b, 139 promoters of which TGFB1I1 (again); group 4a, 44 promoters including TNF and TNFSF10 (again); and group 4b, 65 remarkable promoters. **Conclusions.** The multivariate approach accounts in a systematic way for the variation of the promoter sequences. The major principal components sort promoters according to sequence features, and separate promoters of different functional groups including those involved in the regulation of gene expression in haematopoiesis, particularly the interleukins. The underlying sequence similarities could reflect a common origin for the various genes, i. e. gene duplication followed by divergence, or convergent evolution due to similarities of function. The finding that certain promoters (such as TGFB1I1) were remarkable on more than one dimension invites further study.

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INHIBITION OF THE JAGGED-1/NOTCH PATHWAY INCREASES THE HEMATOPOIESIS-SUPPORTIVE ACTIVITY OF MESENCHYMAL STEM CELLS

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Background and Aims. Mesenchymal stem cells (MSC) are able to support hematopoiesis ex vivo. The aim of this work consisted in determining the contribution of the Notch/Jagged-1 pathway in the ex vivo hematopoiesis-supportive activity of MSC. **Methods and Results.** It is well known that Notch is expressed in CD34+ hematopoietic precursors. By Western blot and flow cytometric analysis, we confirmed the expression of Jagged 1 by MSC. Next, Dexter-type long-term cultures were carried out with MSC and cord blood CD34+ cells in the presence of neutralizing anti-human Jagged-1 antibody (anti-Jagged-1). After 3 weeks, absolute numbers of CD34+, CD10+, CD19+, CD11b+ and CD33+ cells grown in culture were determined by flow cytometric analysis using TruCount tubes. Compared to culture with irrelevant IgG, outgrowth of lymphoid and myeloid cells, as well as expansion of CD34+ cells, were not affected by Jagged-1 inhibition. Repopulation assays in irradiated NOD/SCID mice were set with the expansion product of CD34+ cells co-cultured for one week in contact with MSC and in the presence of anti-Jagged-1 or non- specific IgG. Compared to infusion of CD34+ cells cultured in the presence of control IgG, repopulating activity was increased by Jagged-1 neutralisation. In further experiments, to determine whether this enhancement of repopulating activity was due to direct or indirect effects, the influence of anti-Jagged-1 on MSC was studied. The phenotype, adipogenic, chondrogenic and osteogenic differentiation capacity, as well as CFU-F content of MSC were not affected by Jagged-1 inhibition. However, we noted a 2-fold increase of IL-8 secretion ($p<0.001$) in the presence of anti-Jagged-1. **Conclusions.** These data suggest that, in our conditions, the Jagged-1/Notch pathway inhibits the supportive activity of MSC toward NOD/SCID-repopulating cells. This is not paralleled by changes in the phenotype, differentiation potential or CFU-F capacity of MSC but may be related to inhibition of IL-8 secretion.

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BONE MARROW MESENCHYMAL STEM CELLS IN CHILDHOOD AUTOIMMUNE DISEASES

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Background. Mesenchymal stem cells (MSCs) represent a promising source for cellular therapy due to their easy ex vivo expansion and their capability to differentiate into various cell types of mesenchymal origin. A unique property of these cells is their immunomodulatory and anti-inflammatory function *in vitro* and *in vivo*, making them attractive candidates for the treatment of inflammatory autoimmune diseases. Several clinical trials have been designed in order to study the use of MSCs in the treatment or prevention of acute graft versus host disease (GvHD) exploiting their immunomodulatory capacity. Recently, clinical trials have been conducted with the aim to investigate the therapeutic potential of MSCs in severe, refractory to standard therapy autoimmune diseases (AD). Although the characteristics and the role of MSC have been studied in adults with AD, there are no reports for childhood AD. **Aims.** To study the phenotypical and functional characteristics of bone marrow (BM)-derived MSCs from children with autoimmune diseases. **Patients and Methods.** BM mononuclear cells (MNC) from 11 children with AD, and a control group (CTL) of 9 children with solid tumours without BM involvement, were cultured under conditions that favor MSC growth for five consecutive passages (P1-P5). Cell doubling time (DT), surface antigen expression, CFU-F colony development and differentiation ability towards adipo-, osteo- and chondrocytes were evaluated. BM-MSCs from AD patients and from CTL group were also tested for their ability to suppress *in vitro* proliferation of autologous and allogeneic peripheral mononuclear cells (PBMCs) stimulated with PHA. **Results.** AD-MSCs seem to have similar fibroblast-like morphology, immunophenotypic characteristics and proliferation capacity, as expressed by DT, as those of CTL-MSCs. Their capacity to develop CFU-F colonies and their ability to differentiate into adipo-, osteo- and chondrocytes did not differ from those of the CTL ones. Comparing CTL-MSCs with MSCs from children with

rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), SLE-MSCs appeared to have impaired clonogenic potential. This finding was mainly due to the reduced number of the medium sized colonies (p=0. 006). Both AD-MSCs and CTL-MSCs reduced the proliferation of allogeneic PBMCs in a cell dose-dependent fashion. AD-MSCs inhibited both allogeneic and autologous PBMCs proliferation, after PHA stimulation, although the latter one to a lesser degree (80% vs 57. 5% respectively) without this difference to be statistically significant (p=0. 196). **Conclusions.** BM-derived MSC from children with autoimmune diseases exhibit the same phenotypical and functional characteristics compared with the control group with the exception of the impaired clonogenic potential of SLE-MSCs. Further characterization of the properties of MSC and assessment of their immunomodulatory properties are required before they could be introduced as a therapeutic approach to severe paediatric autoimmune diseases.

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GROWTH PROMOTING AND INHIBITING EFFECTS OF LENALIDOMIDE ON CD34+ STEM AND PROGENITOR CELLS IN VITRO: ROLE OF DOSE AND CYTOKINE SUPPLEMENTATION

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Background. The immunmodulatory drug lenalidomide has emerged as a mainstay in multiple myeloma therapy, and in del(5q) MDS in some countries. However, hematotoxicity and suppression of stem cell mobilization are observed during lenalidomide treatment. It is still a controversial issue to which extent direct effects on hematopoietic stem and progenitor cells (HPC) or indirect modulation of the hematopoietic microenvironment contribute to these side effects. **Aims.** We analyzed the effect of lenalidomide on apoptosis and cytokine-driven proliferation *in vitro* using isolated CD34+ HPC to assess dose dependency, and to distinguish direct from indirect effects. **Methods.** Human mobilized CD34+ HPC were isolated from the peripheral blood. Apoptosis was measured by staining with Annexin-FITC. HPC were stimulated moderately (supplemented with 3 growth factors, 3F = SCF + FLT-3 + IL-6) and intensively (supplemented with 5F = SCF + FLT-3 + IL-6 + IL-3 + G-CSF) with cytokines in serum-free short-term liquid cultures for 7 days. **Results.** Lenalidomide at low to intermediate concentrations (0. 01 - 0. 1μM), significantly increased the percentage of apoptotic HPC after incubation for 24 h with 3F. In the 3F and 5F liquid cultures however, lenalidomide at intermediate concentration (0. 1μM) increased proliferation (3F: 1. 76-fold, 5F: 1. 32-fold increase of the total cell number) and supported differentiation of HPC preferentially into the granulocytic and megakaryocytic (CD41+) lineage. With moderate cytokine stimulation (3F), the CD34+ cell number in the liquid cultures was not increased at low to intermediate concentrations of lenalidomide and reduced at 100μM by 34%. In contrast, expansion of CD34+ HPC with intensive cytokine stimulation was supported by lenalidomid at all concentrations (by up to 85%) except 100μM, where it was reduced by 50%. At 10 μM lenalidomide, also the proportion of colony-forming units, particular erythroid (BFU-E, CFU-E) was reduced. **Conclusions.** Although initially a low rate of apotosis may be observed, at concentrations up to 1 μM proliferation of hematopoietic progenitor cells *in vitro* is not significantly impaired by lenalidomide and may even be increased particularly with multiple cytokine stimulation, resulting in the development of granulocytic and megakaryocytic cells. Only at higher doses (10 μM and more), which are unlikely to be achieved *in vivo* during therapy, lenalidomide shows inhibiting effects on CD34+ HPC. Therefore, indirect rather than direct effects on HPC may contribute to suppression of hematopoiesis as observed during treatment with lenalidomid.

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CORRELATION BETWEEN HAEMOSIDERIN DEPOSITS IN BONE MARROW BIOPSIES AND SERUM MARKERS OF BODY IRON: VALIDATION OF A SEMIQUANTITATIVE METHOD OF ASSESSMENT OF PERLS STAINING

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Background and Aims. Although the detection of hemosiderin pigment deposits (HPD) by Perls staining in bone marrow aspirates is a standardized procedure of diagnostic interest, iron deposits assessment in core biopsy specimens is more controversial. Our aim was to analyze the utility of a semiquantitative method to assess the iron content in bone marrow biopsies, comparing it with known serum markers of body iron: serum ferritin (SF) and transferrin saturation (TS). **Methods.** We examined 75 biopsy samples which were performed in our institution between 2004 and 2009, corresponding to diverse pathology. In addition, simultaneous values of SF and ITS were also collected. Eight of the 75 samples (11%) corresponded to patients with polycythemia vera, in which HPD were absent, and they were considered as negative controls. A subjective semiquantitative assessment with a gradient of 0-4 (from absent to abundant HPD), following the Jakkunen´s criteria for marrow aspirates modiffied by Stuart-Smith et al., was used. The preparations were examined independently by two observers. The results of microscopic examination in the biopsy material were compared with the determination of SF and TS, using the Spearman´s correlation coefficient for statistical analysis. **Results.** Perls staining offered the following distribution in the 75 samples: 7 cases (9%) with traces (grade 1), 23 cases (31%) with low deposits (grade 2), 21 cases (28%) moderate (grade 3) - and 16 cases (21%) with abundant deposits (grade 4). As mentioned above, samples of eight cases (11%) suffering from polycythemia vera, unsurprisingly corresponded to grade 0 (no HPD), being used as negative controls. The degree of coincidence in setting the gradation in each sample by two independent observers was 89%. When compared with serum FT and ST patients, we found a statistically significant correlation: the lowest values of FT and IST were directly correlated with a low degree of HPD and the highest values with a higher degree for the same (p<0. 001, both). **Conclusions.** The grading system used in our study is an adjusted method of assessing the iron deposits in marrow core biopsy; it was also reproducible between different observers. We find a close correlation between these deposits and parameters recognized as the most useful for assessing body iron stores, such as SF and TS.

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IMPAIRED MIGRATION CAPACITY OF GAUCHER DISEASE MONOCYTES

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Background. Gaucher disease (GD) is a genetic lysosomal storage disorder, related to glucocerebrosidase deficiency. Monocytes and macrophages, normally representing the highest glucocerebrosidase activity amongst hematopoietic cells, tend to accumulate high levels of lysosomal glucocerebroside, resulting in infiltration of the reticuloendothelial system, including the spleen, liver and bone marrow, with foamy glucoceribroside-enriched cells. Monocyte recruitment and differentiation play a critical role in defense mechanisms against pathogens and inflammatory response. The chemokine SDF-1 (CXCL12) and its receptor CXCR4 are involved in regulation of migration of multiple cell types, including monocytes. **Aims.** To investigate the migratory capacity of GD-derived monocytes. **Methods.** Peripheral blood mononuclear cells (PBMCs) were isolated from untreated GD patients and healthy volunteers. The study was approved by the Institutional Review Board (IRB) of the Rambam Health Care Campus and all participants signed informed consent. Transwell migration assay was performed in the presence of either 500 or 1000 ng/ml SDF-1. PBMCs were allowed to migrate from the upper chamber through the polycarbonate membrane over 4 hours. Migrating cells were collected from the bottom chamber and were quantified by flow cytometry within the monocyte gate. Peripheral blood serum SDF-1 levels were determined by quantitative ELISA assay (Quntikine® R&D systems). For the evaluation of surface CXCR4 expression, monocytes were stained with CD184-APC conjugated monoclonal antibody. For glucocerebrosidase inhibition, PBMCs derived from healthy volunteers were incubated for 24 h with Conduritol B epoxide (CBE) (Sigma Aldrich, 500μmol/l) prior to detection of monocyte migration capacity. **Results.** Quantitative assessment of monocytes measuring the number of $CD14^+$ cells demonstrated their decreased amount in GD patients (n=14; an average of 5. $5x10^5$ cells/ml) compared with healthy volunteers (n=13; an average of $9x10^5$ cells/ml; p<0. 05). Migration capacity of monocytes obtained from GD patients (n=9) in response to SDF-1, was significantly lower than that observed in monocytes collected in healthy volunteers (n=6), approaching 6. 3% vs. 14%, p<0. 05, for SDF-1of 500 ng/ml, and 9% vs 21. 8%, p<0. 05, using SDF-1of 1000 ng/ml. CXCR4 surface expression on GD-derived monocytes was found to be lower than that of healthy donor-derived monocytes [44. 5% (n=11) vs. 59. 7% (n=7), p<0. 01]. However, serum levels of SDF-1 were significantly increased in GD patients compared to healthy subjects (2603pg/ml, n=10 vs 2039 pg/ml, n=10; p=0. 004). To evaluate the effect of glucocerebrosidase deficiency on monocyte migration, we assessed the influence of CBE, a specific inhibitor of glucocerebrosidase, on healthy monocytes. Initial treatment with CBE resulted in a 60% decrease in monocyte migration capacity in response to 1000 ng/ml SDF-1 compared to untreated cells. **Conclusions.** SDF1-mediated migration of GD monocytes was found to be impaired. This phenomenon can be partly explained by the low surface CXCR4 levels exhibited by monocytes, probably reflecting a negative feedback mechanism to the increased SDF-1serum levels, seen in these patients. Furthermore, the decreased monocyte migration may be related to the inhibition of glucocerebrosidase activity and occurs very early after inducing its blockade.

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INTERLEUKIN-1? PROMOTES THE EXPRESSION OF RECEPTORS FOR THE HEMATOPOIETIC CYTOKINE IN ADULT HUMAN DERMAL FIBROBLASTS

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Background and Aims. In normal hematopoiesis, hematopoietic stem cells and stromal mesenchymal stem cells are reported to express some similar molecules like CD146, and are supposed to have relationship with each other. We recently reported that human interleukin 1β (IL-1β) stimulated bone-marrow stromal myofibroblasts to express a hematopoietic stem cell marker, CD34 (16th EHA, and 53rd ASH meeting). To characterize precisely the effects of IL-1β to the stromal cells, adult human dermal fibroblasts (HDF) were cultured with IL-1β, and the morphological and molecular changes were observed. Materials and **Methods.** HDF were purchased, and cultured in DMEM/F12 medium with 20% KSR with or without recombinant human IL-1β for 3 weeks. Cells were also cultured with IL-1β and recombinant human erythropoietin (EPO). The morphological changes and the expression of specific hematopoiesis-related proteins were analyzed at each 7 day. The concentration of hematopoietic growth factors in the culturing supernatants was also measured. **Results and Discussion.** When HDF were cultured with human IL-1β for 3 weeks, the expression of granulocyte colony-stimulating factor (G-CSF)-receptor, CD13, and SCL increased. When these IL-1β-stimulated cells were cultured in a non-coated dish, cells were floating, and budding of the cells was also observed. When HDF were cultured with IL-1β and EPO, the expression of transcription factor GATA-1 and CEBPA was significantly increased. And CD10, CD20, and CD14 were up-regulated. Vascular endothelial growth factor receptor type-2 also increased to be expressed. In the culturing supernatants IL-6 and G-CSF were secreted significantly. These observations indicate that IL-1β can stimulate to induce HDF toward hematopoietic cells and bone-marrow stromal cells. Now we determine the precise actions of human IL-1β to HDF using NOD/SCID transplantation model *in vivo*.

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PATTERN OF EXPRESSION OF BCL11A IN HEMATOLOGICAL MALIGNANCIES

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Background. The B-cell leukemia 11A gene (*BCL11A*) is essential for normal B and T cells differentiation; it encodes a Krüppel-like zinc-finger protein and functions as a transcriptional repressor through its interaction with several proteins including *BCL6*, *COUP-TF II, SIRT1* and *NuRD* complex. Alternative pre-mRNA splicing of *BCL11A* produces multiple isoforms with different functional features and different subcellular and subnuclear compartmentalization. The relative ratios of *BCL11A* isoforms may determine specific transcriptional regulatory properties and may have a role in pathogenesis of leukemia. **Aims**. Here we evaluated the expression pattern of the *BCL11A* transcripts, XL, L, S and XS, in diagnostic samples from patients with hematologic malignancies in order to elucidate the role of *BCL11A* specific pattern of expression in the pathogenesis of hematologic malignancies. **Methods**. The expression levels of *BCL11A* alternative transcripts have been evaluated in 66 primary samples from patients with lymphoid (18 NHL, 27 CLL), and with myeloid malignancy (10 MPN, 11 CML). Relative quantification with primers specifically designed to detect the *BCL11A* isoforms was performed by real-time RT-PCR in comparison to ABL reference gene on ABIPrism 7500 (PE Applied Biosystems, Foster City, CA). Total RNA from tonsils and pool of non diseased bone marrows were used as calibrators. Expression levels were evaluated with the $2^{-\Delta\Delta Ct}$ method and presented as fold changes. To test differences between groups, Anova and Chi-squared tests were used. **Results**. *BCL11A* gene is expressed in all hematologic malignancies at different levels with the highest expression assessed in CLL samples. XL isoform was detected at levels of 1. 7 and 1. 6 fold changes in NHL and CLL cases, respectively. A 5. 6 fold changes expression level was observed in MPN group and 0. 8 fold in CML patients. Comparable levels of the L isoform was detected in NHL, CLL and MPN. The short isoform S showed a 3. 5-fold change levels in CLL and it was similarly expressed in NHL and MPN groups (with 1. 3 and 1. 55-fold expression changes). CML samples resulted in reduced levels of L and S isoforms with 0. 48-fold and 0. 37 fold change in the 100% of the patients. The medium expression levels of XS variant were of 2. 66-fold changes in NHL, 3. 8 in CLL, 3. 2 in MPN and 1. 2-fold in CML group. Lymphoid and myeloid malignancies showed the following pattern of expression L>XS>XL>S and XL=XS>L>S, respectively. Higher levels of L, S and XS transcripts were observed in the lymphoid with respect to the myeloid group with p values of 0. 02, 0. 03 and 0. 028, respectively. **Conclusions**. This study explores the expression pattern of *BCL11A* isoforms in samples from patients with lymphoid and myeloid malignancies. We found a peculiar expression pattern of *BCL11A* transcripts in CML samples. Results might contribute to the knowledge of the role of the transcription factor *BCL11A* in normal and malignant hematopoietic cells.

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THE EFFECT OF VITAMIN D THERAPY ON HEMATOLOGICAL INDICES IN CHILDREN WITH VITAMIN D DEFICIENCY

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Background. Analysis of the non-classic actions of vitamin D(3) has highlighted a wide range of target tissues for the hormone 1,25-dihydroxyvitamin D(3) [1,25(OH)(2)D(3)]. Systemic or locally produced 1,25 (OH)(2)D(3) may play a role in modulating cell development processes such as hematopoiesis and lymphocyte differentiation. **Methods and Objectives.** We examined the effect of vitamin D3 therapy (10,000 IU/kg, IM) on Red cell indices and total and differential WBCs counts in 25 children (age = 6. 6 +/- 2 years) with vitamin D deficiency (Blood level < 15 ng/ml) before after 4 weeks of therapy. **Results.** There was no significant effect on RBCs count or indices before versus after correction of Vitamin D deficiency. Vitamin D therapy did not have any significant effect on WBC nor on the lymphocyte / neutrophil ratio. There was a small but significant increase in the platelet count after vitamin D therapy. **Conclusions.** In children with VDD, vitamin D therapy did not have significant effect on either RBC or WBC indices.

Table 1.

	Before Vit D	After Vit D
BMI	17 +/- 3.5	17.19 +/- 3.5
Syatolic BP (mm Hg)	106 +/- 11.2	104.6 +/- 11.7
Diastolic BP(mm Hg)	63.1 +/- 5.3	60.56 +/- 4.7
Pulse (/min)	103 +/- 17.7	98.3 +/- 15
25OHD ng/ml	11.2 +/- 4.8	30.8 +/- 13.5
RBCs (million/ul)	4.58 +/- 0.32	4.68 +/- 0.4
Hb g/dl	11.37 +/- 1	11.55 +/- 1
Htc (%)	34.9 +/- 2.3	35.7 +/- 2.7
MCV fl	75.6 +/- 7	75.7 +/- 6.4
MCH pg	24.6+/- 3.22	24.5 +/- 2.5
RDW (%)	15.4 +/-2.7	15.78 +/- 2.9

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PRELIMINARY Results OF A PROSPECTIVE MULTICENTRIC BRAZILIAN STUDY SHOW AN UNBALANCE OF REGULATORY AND EFFECTOR CD4+ T LYMPHOCYTES PRE- AND POST-TREATMENT IN PATIENTS WITH CLASSICAL HODGKIN LYMPHOMA

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Background. Epstein-Barr virus (EBV) can be found latently infecting Reed-Sternberg (RS) malignant cells in approximately 50% of classical Hodgkin lymphoma (cHL) patients in Brazil. EBV signaling leads to an unbalance between effector and regulatory CD4 T lymphocytes in the tumor microenvironment, promoting the immune evasion of RS malignant cells. However, little is known about these lymphocytes subpopulations in the peripheral blood of patients with cHL and how treatment can modify this regulatory/effector ratio. **Aims.** In this study, we analyzed the regulatory and effector $CD4^+$ subpopulations in peripheral blood in patients with EBV related and non-related cHL and the impact of treatment on these cells. **Methods.** This is an open study and, so far, we included 46 patients. We selected the first 14 patients recruited who finished treatment until July 2011. Blood was drawn 1 to 4 months post-treatment. Seven healthy controls were also included in this study. Quantification of regulatory and effector T lymphocytes was done by flow cytometry using CD3, CD8,

CD4, CD25, Foxp3, CTLA-4, GITR, CD127 and interleukin-17 (IL17) antibodies and correlated to phenotypic and clinical parameters in uni- and multivariate models pre and post-treatment. For the present study, only cHL patients whose histology could be confirmed and EBV association established were studied. All patients were HIV negative and received ABVD chemotherapy protocol. **Results.** From the 14 cHL patients selected, 7 were EBV related and 7 EBV non-related. There was an inverse correlation between regulatory ($CD4^+CTLA4^+$ and $CD4^+GITR^+$) and effector T lymphocytes ($CD4^+IL17^+$) (p<0. 001 and p=0. 006, respectively) pre and post-treatment. Also, $CD4^+CD127^+$ lymphocytes were increased post-treatment, without reaching significant difference (p=0,058). $CD4^+CD25^{high}Foxp3^+$ cells were not different pre and post-treatment, although $CD4^+CD25^{high}CD127^{low}$ cells were decreased post-treatment (p<0. 001). When we stratified patients according to EBV presence on RS cells we found that the EBV related cHL group presented with significant difference in $CD4^+CD25^{high}Foxp3^+$, $CD4^+CTLA4^+$, $CD4^+GITR^+$, $CD4^+CD127^+$ and $CD4^+IL17^+$ T cells pre and post-treatment (p=0. 02, p=0. 03, p<0. 001, p=0. 03, p<0. 001, respectively). Interestingly, when we compared the EBV non-related group no difference was found in any $CD4^+$ subpopulations analyzed. **Conclusions.** Our results demonstrate increased regulatory T lymphocytes in cHL patients pre-treatment and an immunological recovery towards effector $CD4^+IL17^+$ T lymphocytes post-treatment. These results also highlight the importance of EBV in Hodgkin's lymphomagenesis and CD4 homeostasis. Further studies investigating the mechanisms in which EBV acts on this regulatory/effector unbalance will contribute not only to our understanding on the pathogenesis of cHL but also to the development of therapeutic strategies designed to manipulate regulatory activity. Given that the incidence of EBV-related cHL, disease presentation and severity are different in developing countries than in developed ones, we emphasize the importance of this ongoing Brazilian multicentric project

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THE PROGNOSTIC SIGNIFICANCE OF BCL-2, TUMOR ASSOCIATED MACROPHAGES AND TOTAL LYMPH NODE INVOLVEMENT BY NEOPLASTIC AND INFLAMMATORY CELLS IN ADVANCED STAGE CLASSICAL HODGKIN'S LYMPHOMA

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Hodgkin's lymphoma (HL) is regarded as the most typical example of a curable cancer. However, current treatment strategies based on risk stratification and response modulation are not precise enough. In addition, the predictive power of biological and morphological parameters is controversial and so far no prognostic models have reached wide acceptance. The aim of this study was to determine whether tissue based variables could add prognostic value to standard clinical parameters and contribute to better risk stratification of HL patients. We analyzed the prognostic relevance of 8 parameters in 85 advanced stage classical HL patients. Univariate analyses showed that Bcl-2 overexpression by HRS cells (>50%Bcl-2+HRS), elevated number of CD68+ tumor associated macrophages (>25%CD68+TAM), IPS>2, and bulky disease were associated with shorter OS (p=0. 007, p=0. 003 p=0. 000, p=0. 002, respectively) and lower EFS (p=0. 031, p=0. 035, p=0. 004, p=0. 014, respectively). Patients with total involvement of the lymph node by neoplastic and inflammatory cells (TLNI) had decreased OS (p=0. 017). Survivin, active caspase 3 and Ki-67 had no significant impact on both endpoints. The multivariate analysis identified >50%Bcl-2+HRS, >25%CD68+TAM, TLNI, IPS>2 and bulky disease as independent prognostic factors for OS (p=0. 025, p=0. 042, p=0. 003, p=0. 000, p=0. 003), and increased CD68+TAM, IPS>2 and bulky disease for EFS (p=0. 044, p=0. 009, p=0. 018), respectively. Based on the cumulative score of unfavorable prognostic factors for OS, a prognostic model was designed stratifying patients into 4 risk groups (low with 0-1, intermediate 2, high 3, very high 4-5 risk factors) and progressively worse 5-year OS (100%, 78%, 45%, 0% respectively, p<0. 001). Our findings support the concept of combining molecular and pathomorphological parameters with clinical data, in order to identify patients who might benefit from more effective primary treatment.

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CD20 REACTIVE INFILTRATE IN CLASSICAL HODGKIN LYMPHOMA: ASSOCIATIONS WITH PRESENTING FEATURES AND CLINICAL OUTCOME

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Background. Several prospective clinical trials using Rituximab to treat classical Hodgkin lymphoma (cHL) patients (pts) are ongoing. The rationale of its use includes Hodgkin and Reed Sternberg (HRC) cells CD20 expression, but also elimination of CD20 positive reactive B cells wich play a role in supporting HRS cells survival and in host immune response suppression. **Aims.** In our study, we evaluated the intratumoral B and T reactive infiltrate and correlated it with presenting features and clinical outcome in high risk cHL. **Methods.** Flowcytomether immunophenotyping of lymph node samples was performed at diagnosis in 11 immunocompetent advance stage cHL pts treated in our institution between January 2010 and December 2011. All pts received ABVD as first line chemotherapy. **Results.** Median age at diagnosis was 38,45 years (range 16-75), 8 pts (72,7%) were male, 3 (27,27%) had a bulky disease, 8 (72,72%) B symptoms, 2 (18,18%) had spleen involvement, 4(36,36%) extranodal disease,5 (45,45%) pts had an IPS score ≥ 3. The histology was nodular sclerosis in 7 (63,63%) and mixed cellularity in 4 (36,36%) pts (Table 1). The mean count of CD20 (CD20+) and CD3 (CD3+) positive reactive cells in our specimens were 14442,6/mL (range 52,3-52080) and 43073,16/mL (range 428-126480) respectively. Using an Anova-one-way analysis, a lower CD20+ mean was observed in pts with IPS≥3, spleen involvement, extranodal disease and B symptoms. In patients with**sym**ptoms, spleen involvement (p<0,05) and extranodal disease, was recorded an higher mean CD3+ to CD20+ cells ratio (ratio). These means differences are not significant at the 0,05 level because of low numbers of pts. At a median follow-up of 11,95 months, 9 pts had reached a complete remission (CR), 2 pts had experienced a relapse/refractory disease and had also a positive PET after two courses of chemotherapy (PET2). A lower CD20+ mean was also recordered in pts with positive PET2 and relapse/refractory disease (p=ns). Moreover the K to Lambda ratio (K/L ratio) was higher in pts with relapse/refractory disease and bulky disease (p<0,05). In one patient with primary chemoresistant disease, the K/L ratio was equal to 8, suggesting the presence of a monoclonal B cells infiltrate associated with poor prognosis. **Conclusions.** CD20+ reactive cells in the tumor microenvironment may be a target in new therapeutic approach and could have a prognostic impact. Moreover many studies describe circulating clonotipic B cells in cHL suggesting they may be the initianing cells for HL. In our experience the presence of a higher K/L ratio is statistically significant associated with relapse/refractory and bulky disease. Further investigations are needed to confirm the prognostic role of CD20+ reactive infiltrate and if flow-cytometric analysis is a usefull tool.

Table 1. Details of patients clinical presenting features.

SUBJECT	SEX	AGE	HISTOLOGY	BOM+	STAGE	IPS	EXTRANODAL DISEASE	BULKY	B SYMP	SPLEEN	PET2
1	M	75	CM		3A	3					POS
2	M	16	SN		2B	3		+	+		POS
3	M	20	SN		2B	2		+	+		NEG
4	M	48	CM		2B	3			+		NEG
5	M	36	CM		2B	2			+		NEG
6	M	41	SN		4BEs	4	LIVER+BONES		+	+	NEG
7	F	24	SN		4AE	1	LUNG	+			NEG
8	M	67	CM	+	4BEs	2	BONES		+	+	NEG
9	F	34	SN		2B	2			+		NEG
10	F	40	SN		2B	2			+		NEG
11	M	22	SN		4AE	4	LIVER				NEG

1403

LYMPHONODE FLOW IMMUNOPHENOTYPE OF CD4+CD8-CD26-CD38+ LYMPHOCYTES IN HODGKIN LYMPHOMA

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Introduction. Hodgkin's lymphoma (HL) is characterized by a small population of neoplastic Reed-Sternberg (RS) cells and extensive infiammatory infiltrate of lymphocytes, plasmacells, eosinophils, and histiocytes similar to that found also in reactive samples. The nature of this infiltrate in HL is largely known: lym-

phocytes surrounding the RS cells are mostly CD4+ T that express activation markers and lack of CD26, an absence potentially relevant to the impaired immune response observed in the clinical course of HL. Flow cytometry (FC) has failed to identify RS cells in HL within the lymphonode biopsy, but it has been occasionally used to examine the lymphocytes surrounding neoplastic cells for distinguish HL infiltrates from reactive lymphoid hyperplasia (RLH) and to predict immune status of such those patients. Our approach was based on the evaluation of FC T CD3+CD4+ in HL infiltrating lymphocytes to compare them to the picture found in RLH and in normal lymphonodes (NL). **Methods.** fresh mechanically disaggregated cell suspensions (10 NL, 65 HL and 52 RLH) were stained with a set of monoclonal antibodies to lymphoid antigens (CD45 pan-leukocytes, CD19 for B lymphocytes, T-cell antigens such as CD3, CD4, CD8 and CD38 as markers of activation and CD26) and then analyzed by FC. **Results.** HL is richer in T-cell lymphocytes than RLH (CD3/CD19 2,9 vs 1,8) and in both groups CD4/CD8 ratio (respectively 6,5 and 6,9) is higher than in NL (CD4/CD8<3) . CD4+CD26+ is higher in NL and RHL, while is much lower in HL (respectively 70, 50 and 20; p < 0,0005). CD4+CD38+ was also higher in HL (43+17) compared to RLH (18+12). When we have analyzed together $CD4^+CD26^-CD8^-CD38^+$, the difference between HL (38%) and RLH (9%) was significantly different (p < 0,001). **Conclusions.** Since a $CD4^+CD26^-CD8^-CD38^+$ profile appears restricted to infiltrate in HL, immunophenotyping may represent a useful tool for speedy diagnosis (or suspect) and the detection of the anergic status in patients with Hodgkin lymphoma.

1404

OUTCOME AND PROGNOSTIC FACTORS IN PATIENTS WITH HODGKIN LYMPHOMA (HL) WHO REMAIN PET/CT-POSITIVE AFTER ABVD COMBINATION CHEMOTHERAPY: POTENTIAL APPLICATIONS FOR THE DESIGN OF SUBSEQUENT TREATMENT

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Background. Patients with HL who remain PET/CT(+) after ABVD combination chemotherapy have a relatively unfavorable prognosis. Prognostic factors for the outcome of PET/CT(+) patients, especially after additional radiotherapy, have not been systematically studied. However, such factors may have a role in decision making between additional RT vs. salvage chemotherapy and autologous stem cell transplantation (ASCT). **Aims.** We aimed to identify predictive factors for the outcome of HL patients who remain PET/CT(+) after ABVD chemotherapy and are planned to receive additional RT. **Methods.** 311 patients with HL were treated with 4-8 cycles of ABVD from December 2004 to early 2011: 245 responders (CR, CRu, PR) underwent PET/CT after ABVD; 38 responders did not due to technical reasons. There were missing data for 6 patients, 3 died prematurely and 19 rapidly developed progressive disease prior to PET/CT assessment. Among 245 responders, 52 (21%) were PET/CT(+) and were retrospectively analyzed in terms of disease progression. **Results.** Characteristics of the 52 PET/CT(+) patients were as follows: median age 31. 5 years (15-73), 58% males, 77% nodular sclerosis, 27% stage III/IV, 19% B-symptoms, 10% ≥5 involved sites, 31% anemia, 18% significant leukocytosis, 10% severe lymphocytopenia, 56% ESR ≥50 mm/h, 31% elevated LDH, 39% albumin <4g/dL, 23% IPS≥3. The median SUVmax was 5. 2 (IQR 3. 7-8. 5) without any correlation with the above parameters. The majority of patients were irradiated (42/52 or 81%; median dose 3620 cGy; range 2880-4600). Ten (10) patients were not irradiated (too extensive disease 2, rapid progression 1, medical decision 1, refusal 1, findings judged borderline by treating physician 5). After a median follow-up of 29 months (2-67), 22/52 patients have experienced disease progression. The median SUVmax was 6. 7 versus 4. 1 for patients with and without disease progression respectively (p=0. 02). Progression free survival (PFS) was 73%, 63%, 51% and 46% at 1, 2, 3 and 4-5 years after PET/CT respectively. The 3-year PFS was 59% vs. 24% for patients with SUVmax < and ≥9 respectively (p=0. 0005). Among other factors, only anemia significantly affected PFS in univariate analysis (p=0. 01). In multivariate analysis, SUVmax ≥9 was the only independent prognostic factor for PFS (hazard ratio 3. 1; 95% CI: 1. 1-9. 2; p=0. 037) obscuring the significance of anemia (p=0. 17). The significance of SUVmax ≥9 was maintained, if the analysis was restricted to the 42 irradiated patients (3-year PFS 69% vs. 30%, p=0. 004). In 14 of the irradiated patients, a repeated PET/CT scan converted to negative: only 2/14 have relapsed for a 3-year PFS of 83%. **Summary and Conclusions.** Despite additional radiotherapy, patients with HL who remain PET/CT(+) after ABVD have a 50-60% risk of relapse over the next 4 years. Patients with lower SUVmax (optimal cut-off to be determined) may benefit more from additional radiotherapy, while more intense uptake was associated with an even higher risk of disease progression. These findings may facilitate the selection of optimal treatment in this patient subgroup with uncertain prognosis. The potential contribution of conventional prognostic parameters needs further investigation. Patients rendered PET/CT(-) after RT appear to be at lower risk of relapse.

1405

BRENTUXIMAB VEDOTINE IN HEAVILY TREATED HODGKIN LYMPHOMA, A RETROSPECTIVE SINGLE CENTER STUDY ON 30 PATIENTS

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Hodgkin lymphoma (HL) is a curable disease in up to 80% of patients but for the remaining relapsed patients after autologous stem cell transplantation (ASCT) no standard therapy is available. Anaplasic large cell lymphoma (ALCL) patients in relapse after CHOP have also a poor outcome. CD30 is expressed on the surface of HRS cells and in ALCL. Minimal clinical activity has been reported with unconjugated anti-CD30 antibodies. To enhance antitumor activity, the antitubulin agent monomethyl auristatin E was attached to the CD30-specific monoclonal antibody producing the antibody-drug conjugate brentuximab vedotin (BV). The phase I showed promising results (Younes NEJM 2010) and high reponse rates in two phase II studies both in HL & ALCL (Advani & Chen ASH 2010). **Aims**. This retrospective study was designed to evaluate the activity of single-agent BV in unselected rel/ref HL & ALCL in a single center given with the same indication as the phase II. Our primary outcomes are response rate, toxicity and, progression free survival (PFS) (calculated from the first dose of BV to progression). PATIENTS and **Methods**. From July 2009 to july 2011, 30 patients (14M/16F) with a median age of 31 years (19 -69y) received at least one dose of BV for 20 HL & 10 ALCL. Nine patients were in protocols and21 inthe name patient program . First line chemotherapy (CT) was ABVD in 17 cases and CHOP in 7, 11 patients were primary refractory and 7 patients received radiation therapy (RT). The median number of previous CT lines was at 4 (1 - 8) and 20/20 patients with HL received ASCT (two times in 10 cases) and 2 allotransplant . Before the first dose of BV, 21/30 patients were “on”chemotherapy and 9 had relapsed after response. Response was evaluated after 4 cycles with PET in 16 cases. **Results**. Two patients with refractory ALCL received only one course of BV and died rapidly from progressive disease. 28 patients are evaluable for response and PFS. The median number of cycles was 7 (1-16) and 5 patients are remaining on therapy. Haematological toxicity was mild with no transfusion and no growth factor support. 3 patients had grade 2 sensitive neuropathy, one hepatic toxicity rapidly reversible and one pulmonary toxicity when given shortly after mediastinal RT. The maximal response was CR/CRu in14 ptsand 4 PR with an overall response rate of 64% with higher CR rates in ALCL. The median PFS was at 8 months (range 3 -34+) and four patients with ALCL had received subsequent consolidation (ASCT n = 3 & NMT n =1), only 3 patients with HL had an allogeneic donor and none were able to receive the transplant. At the stopping date (December 2011), 6 patients had died from the disease and 13 had received further therapy. **Conclusions.** BV is effective in this heavily pretreated group of patients (mostly end-stage HL) even if the PFS is short because many responding patients relapsed after and all HL were heavily pretreated with 50 % having received two transplant.

1406

ABSOLUTE MONOCYTE COUNT AND CHEMORESISTANT HODGKIN'S LYMPHOMA

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Background. It has recently been described an association between a high absolute monocyte count (AMC) and low absolute lymphocyte/absolute monocyte ratio (LMR) in peripheral blood at diagnosis with inferior survival in Hodgkin's Lymphoma (HL) (Porrata *et al*. 2012). A connection between an

increased number of tumour-associated macrophages (TAM) and treatment failure had already been established (Steidl *et al.* 2010). Theoretically, the AMC is an estimate of the TAM. **Aims.** To correlate chemoresistant disease (CRD) to these new biomarkers and other well-known HL prognostic factors. **Methods.** We accessed the available full blood count at diagnosis and medical records of 95 patients with HL, negative for human immunodeficiency virus, diagnosed in our center from 2000 to 2010. We defined CRD as failure of first-line chemotherapy, high AMC as ≥ 900/mm^3 and low LMR as <1. 1. **Results.** The median age at diagnosis was 30 years-old [15;83] and 54. 7% of the patients were male. The median follow-up was 58 months [1;131]. All patients were treated with either ABVD (92) or BEACOPP (3) regimens, based on Ann Arbor staging system and International Prognostic Score (IPS) stratification, with or without radiotherapy. Eighteen (18. 9%) patients were submitted to autologous stem cell transplantation (ASCT) due to CRD (11) or relapse (7). Nine (9. 5%) patients died from disease progression/relapse (6), non-lymphoma related causes (2) and secondary neoplasm (1). Fifteen (15. 8%) patients had CRD after first-line treatment: 11 were submitted to ASCT, nine (81. 8%) of which achieved complete remission (CR) and 2 died prior to the 100th day of ASCT; 1 died before ASCT was performed; and 3 are currently receiving second-line treatments. Within the CRD group, thirteen (86. 6%) patients had high AMC (8), low LMR (2) or both (3). Eleven (52. 3%) out of 21 patients with high AMC had CRD, and seventy (94. 6%) out of 74 patients with low AMC did not (p<0. 0001). The mean AMC was superior (1. 00 vs 0. 64; p<0. 0001) for the CRD patients. CRD was also associated to advanced stage disease (III/IV) (p=0. 008), high white blood cell count (≥15x10^9/L) (p=0. 019), low LMR (p=0. 016), IPS ≥3 (p=0. 014) and hypoalbuminemia (<40g/L) (p=0. 04); but not with sex, age, histological classification, absolute lymphocyte count, haemoglobin or LDH levels at diagnosis. On multivariate analysis, AMC ≥ 900/mm^3 was highly and independently associated with CRD (OR: 16. 4[2. 8; 94. 2]; p=0. 002). Out of the 11 patients who relapsed from CR, only two (18. 2%) had high AMC (1 also relapsed after ASCT, and 1 other died). As expected, there was worse 5-year overall survival (OS) in patients with CRD (77. 5% vs 94. 2%; p=0. 048) and high AMC (78. 5% vs 95. 1%; p=0. 01) but no differences in progression-free survival (PFS). **Conclusions.** In our group of patients, a high AMC was strongly associated with CRD, but not with relapse from CR. Most of these chemoresistant patients, however, respond to ASCT, hence the lack of impact on PFS. Although this is a small series, we suggest that AMC may predict which patients will not respond to standard fist-line chemotherapy.

1407

ANALYSIS OF BONE MARROW REACTIVE CHANGES AND PET/CT DIFFUSE BONE MARROW UPTAKE IN STAGING OF HODGKIN LYMPHOMA

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Background. Bone marrow aspirate (BMA) and bone marrow biopsy (BMB) are usually performed in the staging assessment of Hodgkin Lymphoma (HL) patients. Despite a low incidence of bone marrow infiltration (BMI), a high percentage of patients is characterized by non-specific lesions, that could be related to high level of serum cytokines. These non-specific lesions indicate a reactive condition that is likely to account for the FDG-diffuse bone marrow uptake (BMU) frequently observed at initial staging imaging and that does not seem to be related to malignant involvement. **Aims.** To evaluate the significance of bone marrow reactive changes and FDG-diffuse BMU in initial staging of HL. **Methods.** 92 HL patients (median age 33 years, range: 16-79, 44 male, 48 female) undergoing staging BMA, BMB and PET/CT were included in this retrospective study. BMA and BMB data were obtained from the files of the Hematology and Pathology Units and reviewed by expert pathologists. PET/CT results were reviewed by a Nuclear Medicine expert. BMU was assessed visually according to liver uptake (0=below liver uptake, 1=corresponding to liver uptake, 2=above liver uptake), as previously described (Salaun PY et al., Eur J Nucl Med Mol Imaging, 2009). Clinical and laboratory characteristics were collected. **Results.** 92. 4% of patients had classical HL and 7. 6% had nodular lymphocyte predominant HL. 65. 2% had advanced stage disease. In 32. 6% of cases IPS was equal or higher than 3. BMB was positive for BMI in 6. 5% of patients, whereas focal skeletal FDG-uptake was seen in 9. 8%. BMB and PET/CT showed concordant results in 90. 2% of cases. BMA showed hypercellular marrow in 44. 6%, disgranulopoiesis in 61. 9%, myeloid hyperplasia in 5. 4%, eosinophil hyperplasia 29. 3%, dyserythropoiesis in 8. 7%, erythroid hyperplasia in 15. 2%, reactive lymphocytosis in 4. 3% and higher values of plasma cells in 10. 9% of smears. Overall bone marrow biopsy cellularity was 60. 2%, significantly higher than that of a control group (48%, p=0. 01). There was no statistical difference between the age distribution in the patient group and the controls (p=0. 8). Diffuse BMU was detected in 51. 6% of scans (grade 0: 8. 3%, grade 1: 29. 2%, grade 2: 62. 5%). Univariate analysis found a correlation between BMU and bone marrow biopsy cellularity (p=0. 05), hypercellular marrow at BMA (p=0. 02), myeloid hyperplasia (p=0. 003), erythroid hyperplasia (p<0. 001), WBC values (p=0. 02) and CRP level (p=0. 05). No statistical link was found between BMU and other factors analysed, in particular BMI, detected either by BMB or by PET/CT. After a median follow up of 36 months (range 7 - 88), the 2-year OS and PFS were 93. 1% and 82. 4%, respectively. By univariate analysis diserythropoiesis (p=0. 03), erythroid hyperplasia (p=0. 01), elevated CRP level (p=0. 03), PET-2 results (p=0. 07) and advanced stage (p=0. 04) were predictors of lower PFS, whereas only a positive PET-2 displayed an independent prognostic significance in the multivariate analysis (p<0. 001). Diserythropoiesis showed a trend toward significance (p= 0. 06, see Figure 1). **Conclusions.** Diffusely increased BMU is frequently seen at initial staging in HL patients and it is due to reactive changes seen at BMA and BMB. BMA findings, such as diserythropoiesis, are predictors of outcome.

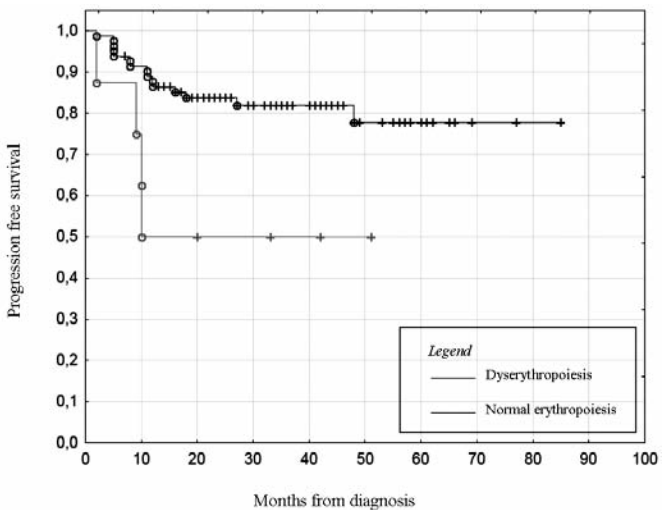

Figure 1.

1408

INTRATECAL LIPOSOMAL CYTARABINE AS CNS INVOLVEMENT PROPHYLAXIS IN DIFFUSE LARGE B CELL LYMPHOMA. THE SPANISH EXPERIENCE

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Introduction. Lymphomatous meningitis (LM) in patients with Diffuse Large B Cell Lymphoma (DLBCL) is usually an early complication and with poor prognosis. Risk factors have been previously identified for this complication. DepoCyte is an extended released liposomal cytarabine formulation (LC) which has demonstrated better efficacy compared to standard cytarabine for the treatment of LM in one randomized clinical trial. **Aims.** The aim of this study is to evaluate efficacy and safety of intrathecal LC as prophylaxis of LM in DLBCL patients at high risk of CNS involvement. **Methods.** A retrospective study was carried out on 26 Spanish sites including patients diagnose with DLBCL and at risk of LM - defined by the presence of at least one of the following: retroperitoneal mass ≥10 cm; Waldeyer ring, sinus, vertebral or bone, and testicular involvement; LDH more than twice the upper normal limit; bone marrow involvement > 30% and serology VIH+. All patients had received LC as IT prophylaxis for LM in the period April 2005 - June 2011. Main endpoints were effectiveness (leptomeningeal involvement rate) and safety of the IT prophylaxis. **Results.** Data from 135 patients were analyzed. Baseline characteristics were: Mean age 57 ± 16 years (range: 18-80 years). Males/Female: 89/46;Ann Arborstage IV 71%. Systemic chemotherapy was the standard of the different

hospitals, all alkylating based regimens, being R-CHOP the most frequent one (96%). LC was administered as intrathecal prophylactic treatment for LM in all patients in doses of 50 mg. 124 patients completed the IT prophylaxis treatment with a mean of 2. 6 ± 0. 9 (range: 1-6) administrations of LC (11 patients died before finish prophylaxis), and with a median follow up of 19 months (range: 3-60 months) we reported no cases of LM. Just two patients (1. 48%) had CNS progression, both developed CNS parenchymal mass at the same time of systemic progression. We reported no adverse event in 89 (66%) patients. All adverse events were reversible, being headache the most common toxic effect 39 (29%) cases, and only 10% of patients had grade III or IV toxicity. Chemical arachnoiditis prophylaxis was given to 107 patients. **Conclusions.** This retrospective study shows that CNS prophylaxis with intrathecal LC in DLBCL patients at high risk of CNS involvement is effective and well tolerated.

1409

GEMCITABINE, DEXAMETHASONE AND CISPLATIN IS EFFECTIVE AS A SALVAGE CHEMOTHERAPY REGIMEN IN RELAPSED OR REFRACTORY HODGKIN'S DISEASE PRIOR TO ASCT

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Background. patients with refractory or relapsed Hodgkin's lymphoma are best treated with an approach involving high-dose chemotherapy and autologous stem cell transplantation (HDT-ASCT) However, in the absence of randomized trials of salvage regimens, the standard salvage option is unknown. In this context, the choice of a salvage regimen is guided by efficacy and toxicity. **Patients and Methods.** We retrospectively reviewed the data of our 33 consecutive patients with relapsed or refractory Hodgkin's lymphoma (median age 40, range 19-68) treated with the GDP regimen (gemcitabine 1000 mg/m2 on day 1 and 8, dexamethasone 40 mg orally days 1-4 and cisplatin 33 mg /m2/day days 1-3). Response was defined according to the IWG 2007 criteria, using PET-scan evaluation. **Results.** The overall response rate (CR + partial remission) was 63%. Eighteen (54,5%) patients achieved complete remission; three (9 %) partial remission and twelve (36,36%) patients had stable disease or progression . Twenty-two patients (66,6%) underwent HDT-ASCT. With median follow-up of 5 years, the OS was 82% for patients with complete or partial remission, and 41 % for patients with progressive disease (p = 0. 15). Age < 45 years, treatment with HDT-ASCT, and response to GDP was associated a significantly longer OS (p< 0,0001; p=0,001; p=0,015,respectively). **Conclusions.** GDP is a active regimen that compares favourably with previously published salvage regimens. Further prospective randomized studies in the setting or relapsed/refractory regimens would greatly help the therapeutic choice in this difficult patient population.

1410

PROGNOSTIC VALUE OF PRETRANSPLANT FDG-PET IN PATIENTS WITH REFRACTORY AND RELAPSED HODGKIN LYMPHOMA TREATED WITH HIGH-DOSE CHEMOTHERAPY AND STEM CELL TRANSPLANTATION

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Autologous stem cell transplantation (ASCT) is considered the standard salvage therapy for relapsed or refractory Hodgkin disease (HD). Recently publications have confirmed the role of 18F-fluoro-deoxyglucose positron emission tomography (FDG-PET) about the predictive value before ASCT. In this retrospective study we want to assess the predictive value of FDG-PET performed before ASCT for the clinical outcome such as progression-free survival (PFS) and overall survival (OS) in pts with HD. Between January 2005 and August 2011 in our Bone Marrow Transplant Unit were performed 41 autologous stem cell transplantation (ASCT) in patients with relapsed or refractory Hodgkin lymphoma. The median age was 31 years (range 18-58 years); at time of transplant 21 pts (51%) were in complete remission (CR), 5 pts (12%) in partial remission (PR) and 15 pts (37%) with refractory disease. The FDG-PET before ASCT was negative in 21 pts (51%) and positive in 20 pts (49%). After a median period of observation of 24 months (range 0-76 months) the overall survival (OS) was 94% in the FDG-PET-negative group and 31% in the FDG-PET positive group (p: 0. 0002). The progression free survival (PFS) was 95% and 33% (p: 0. 000) respectively for pts with FDG-PET negative and positive after a median time of observation of 14 months (range 0-76 months). After three months form ASCT 37 out 41 patients performed FDG-PET for restaging, the FDG-PET was negative in 26 pts (70%) and positive in 11 pts (30%). The PFS was 88% in the FDG-PET-negative group and 10% in the FDG-PET positive group (p: 0. 0000). The OS was 90% and 37% respectively in FDG-PET negative and FDG-PET positive scans (p:0. 0008). Sixteen out 20 pts FDG-PET pretrasplant positive performed an FDG-PET after transplant resulted negative in six pts (37. 5%). In multivariate analysis the FDG-PET pre ASCT and status disease pre-ASCT improved progression free survival (p 0. 000). Our results confirm that in patients with relapsed/refractory HL a negative pre-transplant FDG-PET is associated with a better OS and PFS. About one third of patients with a positive pre-transplant FDG-PET could be recovered by high dose therapy and transplant. Patients with persistent positive FDG-PET after transplantation are associated with a poor prognosis and should be considered for tandem ASCT, allotransplant or new drugs.

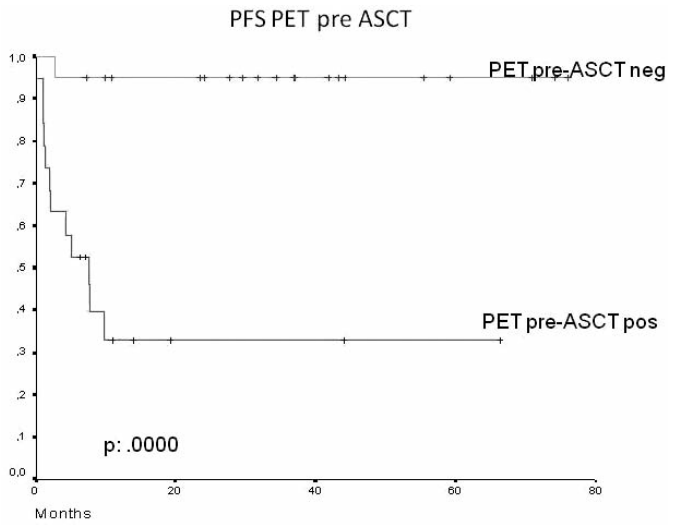

Figure 1.

1411

FEASIBILITY AND EFFICACY OF BCVPP REGIMEN IN VERY ELDERLY PATIENTS WITH HODGKIN'S LYMPHOMA. A SINGLE CENTER EXPERIENCE ON NINETEEN PATIENTS

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Background. While some age-adapted modifications of ABVD regimen have been proposed for elderly Hodgkin's lymphoma (HL) patients (pts), no specific chemotherapy regimens have yet been recommended for very elderly or elderly pts with severe comorbidities. The five-drug-combination BCVPP (Carmustine, Cyclophosphamide, Vinblastine, Procarbazine and Prednisone) has the advantage of avoiding anthracyclins and being potentially effective and tolerated in HL pts. **Aims.** We report on the therapeutic efficacy and tolerability of BCVPP regimen in a group of very elderly (≥80y) and elderly pts with severe comorbities unfit for anthracyclin-containing chemotherapy. **Methods.** Eligibility criteria included age older than 80, severe comorbidities (one grade 4 or at least 3 grade 3 according to Cumulative Illness Rating Score for Geriatrics - CIRS-G), relapse/refractoriness to ABVD or other regimens. BCVPP (Carmustine 100 mg/sqm iv d 1, Cyclophosphamide 600 mg/sqm iv d 1, Vinblastine 5 mg/smq iv d 1, Procarbazine 100 mg/sqm d 1-10 po, Prednisone 60 mg/sqm d 1-10 po) was administered every 42 days for a maximum of 6 cycles. Reduction of the doses was applied for adverse events and intolerance. **Results.** Nineteen pts have been treated (M/F ratio 1/1. 8). Median age was 77y (65-88). Eleven pts were unfit for dementia (1), grade-3 comorbidity (4) or grade-4 comorbidity (6) according to CIRS-G, 8 were aged ≥80y. Sixteen pts were treated upfront, while one was refractory/intolerant to low dose ABVD, one relapsed after ABVD and MOPP and one after radiotherapy for localized HL. Histology was nodular sclerosis in 10 pts, mixed cellularity in 5, lymphocytic depletion in 1 and classic NOS in 3. EBV LMP-1 was detected by immunohistochemistry in RS cells of 4/7 cases. Fourteen pts (74%) had advanced disease (7 stage III and 7 stage IV). Eleven pts (58%) presented with B symptoms and 12 (63%) with International Prognostic Score>2. The median number of cycles administered was 5 (range 1-6). In 11/19 (58%) pts doses were reduced. The mean administered dose of any drug was >75%. Hematologic grade 3-4 toxi-

city was seen in 8 (42%) pts (neutropenia in 7 and neutropenia and thrombocytopenia in 1). Nine pts experienced infectious complications: 5 pneumonia, 4 FUO, 1 CMV reactivation, 1 soft tissue infection and 1 disseminated HZV infection with meningoencephalitis 6 and 11 months after the end of therapy respectively. One pt died of ischemic stroke and 1 was lost to follow-up after 1 cycle. Of the 17 evaluable pts, ORR was 94% (12 CR/Cru, 4 PR, 1 NR). Relapse occurred in 4 pts after a median of 22 months (17-49). Three pts died of lymphoma, 4 of toxicity (3 pneumonia and 1 carmustine-related interstitial lung fibrosis), 2 of unrelated causes (1 ischemic stroke and 1 respiratory distress). Ten are alive (2 with progressive disease) after a median follow-up of 20 months (2-171). The actuarial 3-year PFS and OS were 39. 4% and 54% respectively. Our data are similar to those published in a recent series of elderly but younger pts. **Conclusions.** BCVPP regimen provides an effective and feasible therapeutic option for very elderly patients with HL.

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RELAPSED OR REFRACTORY (REL/REF) HODGKIN LYMPHOMA (HL) POST-AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) - A RARE CANCER WITH NO STANDARD OF CARE

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Background. HL is the most common CD30-positive malignancy and accounts for ~10% of all lymphomas. Rel/ref HL post-ASCT is extremely rare, occurring in only 3-9% of HL patients in EU5. As a result of the small patient numbers, research into the epidemiology and disease burden of rel/ref HL post-ASCT is very limited. **Aims.** To describe the burden of HL post-ASCT in terms of its epidemiology and humanistic and economic burden, as well as to present the current treatment options for patients in this setting. **Methods.** A literature search for HL epidemiology data was conducted. A systematic literature review of clinical effectiveness and safety of available therapies in rel/ref HL post-ASCT was conducted in Medline/Medline in Process, Embase and the Cochrane Library, and targeted searches were conducted for HL clinical guidelines from institutions and organizations. Systematic reviews of economic and health-related quality of life studies in HL were also performed. **Results.** In the EU and European Economic Area the prevalence of HL has been estimated to range from 0. 017% to 0. 035%. This rate is below the orphan designation threshold in the EU (prevalence <5 cases/10,000 population). In EU5 countries there are an estimated 500 patients per year with rel/ref HL post-ASCT who would be eligible for further treatment. There is currently no consensus regarding recommendations from clinical guidelines for the treatment of patients with rel/ref HL post-ASCT. Current options, which include chemotherapy and allogeneic stem cell transplant (allo-SCT), are associated with high rates of toxicity or are limited by high-risk of treatment-related mortality and donor availability. Recent evidence suggests that 27-36% of patients with rel/ref HL post-ASCT receive allo-SCT compared to only 6-8% who receive a second ASCT. The median overall survival (OS) for patients who relapse following, or are refractory to, ASCT is 2. 4 years, while outcomes for patients who are refractory to ASCT or relapse within 1 year of therapy are particularly poor (median OS 1. 2 years). HL is the second most costly cancer overall, and has been estimated to be the most costly cancer in men and women of working age. HL affects adults who are economically active, with a median age at time of diagnosis of 38 years. As a result, the impact of lost productivity is likely to be substantial. In terms of the humanistic burden, many rel/ref HL patients suffer from debilitating B-symptoms, which include fever, night sweats and weight loss. The presence of B-symptoms is a marker for more advanced disease as it indicates systemic involvement. HL patients report chronic fatigue, decreased social, emotional and physical functioning, increased psychological distress, decreased sexual function and general health, and an impaired ability to carry out everyday activities. **Conclusions.** Rel/ref HL post-ASCT is rare, with no standard of care. These patients have a poor disease prognosis along with a significant humanistic and economic burden. Therefore, effective treatment approaches with a good safety profile are required.

1413

RESPONSE TO SALVAGE CHEMOTHERAPY CONDITIONS OUTCOME OF PATIENTS WITH HODKGIN'S LYMPHOMA SUBMITTED TO HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS TRANSPLANTATION

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The majority of patients with Hodgkin's Lymphoma (HL) has sustainable complete remission with first-line chemotherapy. However, some patients relapse after therapy and pose a significant therapeutic challenge. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is a recognised second line therapy for HL. This retrospective study aimed to review the outcomes of ASCT in relapsed/refractory HL patients and the role of salvage therapy before ASCT. Data was collected from 41 consecutive patients that underwent ASCT between 1999 and 2011. All patients had the diagnosis of relapsed/refractory HL. The histological HL classification was Nodular Sclerosing (86. 2%) or Mixed-Cellularity (13. 8%) subtype and the majority (66%) had an Ann Arbor stage III or IV, at time of diagnosis. ESHAP (82. 4%) or ICE (17. 6%) was the salvage chemotherapy given. The overall response rate (ORR) after treatment was 86. 8% (84. 6% with ESHAP and 91. 6% with ICE, p=. 488) and 13. 2% had refractory disease. Subsequently, all patients were treated with high-dose chemotherapy (BEAM) followed by ASCT. The median age at transplant was 31(range:21-67). Median time to neutrophil and platelet recovery was 10 and 13 days, respectively. At day +100 post ASCT, two patients (4. 9%) maintained refractory disease and two patients (4. 9%) had died of sepsis - all these patients presented progressive disease at time of ASCT. The median follow-up was 29 months. The 5-year overall survival (OS) was 60. 3% +/-9. 6% (74. 2% for ESHAP-treated patients) and 5-year progression free survival (PFS) was 64. 8% +/-8. 3% (77. 3% for ESHAP-treated patients). Refractory disease at time of transplant was associated with significantly worse OS (5-year OS: chemo-sensitivity 63. 7% vs. refractory-disease 40%;p=. 010). On univariate analysis, no significant differences were observed regarding OS of gender, age, stage, time from diagnosis to ASCT (<12 vs. >12 months) and salvage chemotherapy regimen (ESHAP vs. ICE)In summary, ORR after salvage chemotherapy was high and this therapeutic approach followed by BEAM-ASCT is associated with a favourable 5-yr OS rate. No significant difference was observed between patients treated with ESHAP or ICE although there is a trend for better OS and PFS in ESHAP-treated patients. The optimal choice of salvage therapy remains unclear, because different regimens have not been directly evaluated in randomized trials. The ideal salvage regimen should produce a high response and not impair the ability to mobilize and collect peripheral blood stem cell for ASCT. Based in our experience, patients who had refractory disease at time of transplant might not benefit of high dose chemotherapy and ASCT; other novel therapeutic options should be pursued for this small group of patients.

1414

LONG-TERM OUTCOME OF ADOLESCENTS AND YOUNG ADULTS WITH HODGKIN LYMPHOMA TREATED IN ACCORDANCE TO THE MODIFIED PEDIATRIC PROTOCOL DAL-HD-90: A SINGLE CENTER EXPERIENCE IN RUSSIA

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Background. About 3200 new cases of Hodgkin lymphoma (HL) diagnosed every year in Russia. HL occurs in all ages but adolescents and young adults (AYA) are most often affected. The standard of care for adolescents is undefined. Currently, they are inconsistently treated with either pediatric or adult protocols. The information about treatment of young adults with pediatric protocols is missing. **Aims.** The aim of this study was to evaluate the long-term results of therapy of AYA with HL by dint of the pediatric protocol in our center. **Methods.** During 03. 1995-12. 2009 in this study 99 (m-38, f-61) patients with median age 19. 2 years (range 15-29) with de novo HL was enrolled. All patients were treated according to the modified pediatric protocol DAL-HD-90 which is a combined-modality treatment regimen. Patients are allocated to three treatment groups (TGs) based on early- (TG1, n=11), intermediate- (TG2, n=34), and advanced-stage (TG3, n=54) disease. Involved field radiotherapy (IFRT) follows after a chemotherapy. The original protocol is modified in the following positions: (1) oral procarbazine was replaced by intravenous dacarbazine which was administered at 1 and 14 days at a dose of 375 mg/m^2; (2) young adults received vinblastine vice vincristine; (3) all patients with advanced stages of HL receiving 2 cycles of ODPA independently from gender, and (4) doses of IFRT were increased from 20-25 Gy to 30 Gy. The subjects with a poor response after 2 cycles of ODPA (stabilization or disease progression) have changed treatment on more intensive adult schedule of BEACOPP escalated. This change of therapy was considered as an event in the calculation of event free survival (EFS). The cutoff for data analysis was 12. 2011. EFS and overall survival (OS) were analyzed using the Kaplan-Meier method and the log-rank test. **Results.** Forty-nine adolescents (median - 16. 5, range 15-18 years) and 50 young adults (median - 19. 6, range 19-29 years) were available for the analysis. Median follow-up time was 9. 5 years. One patient lost to follow-up. Seven patients died

and all deaths were related to HL progression or relapse. Second malignancies occurred in 2 girls (ovarian and thyroid carcinomas). The probabilities of EFS and OS at 10 years (10y) were 91±9% and 91±9% (TG1), 88 ± 6% and 94±4% (TG2), and 73±7% and 92±4% (TG3), respectively. The probabilities of 10y-EFS in TG(1+2) patients were 89±5% versus 73±7% in TG3 patients (P=0. 095). As a result of poor early response after two cycles of ODPA the therapy was changed on the adult schedule BEACOPP escalated for 7 patients (TG1-1, TG2-1, TG3-5). Five patients in this group are alive with continuous complete response (CCR). Ten-year EFS and OS rates did not differ significantly between adolescents and young adults: EFS - 82±6% and 78±6%, respectively (P=0. 54); OS - 92±4% and 94±4%, respectively (P=0. 72). **Conclusions.** Our data confirm the rationality to use pediatric protocols in adolescents up to 18 years with HL. The outcome for young adults did not fundamentally different from that of adolescents.

1415

IPHOSPHAMIDE, GEMCITABINE, VINORELBINE (IGEV) CHEMOTHERAPY PLUS AUTOLOGOUS STEM CELL TRANSPLANTATION AS SALVAGE TREATMENT OF RELAPSED/REFRACTORY HODGKIN LYMPHOMA PEDIATRIC PATIENTS

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Background. Although most patients with Hodgkin Lymphoma (HL) will be cured with initial therapy, approximately 10-25% of patients relapse after first-line therapy or are primary refractory. Because of lack of randomized studies comparing various salvage regimens, it is not possible to identify one as superior to another. Thus, it is critical to select a salvage regimen that has the potential to induce a high response rate with low nonhematologic toxicity. **Aims.** To retrospectively evaluate the outcome of children affected by relapsed/refractory HL treated with salvage treatment consisting of IGEV chemotherapy plus a*utologous peripheral* blood *stem cell* transplantation (APBSCT) in our Institution. **Methods.** Pediatric patients, previously enrolled in AIEOP (Associazione Italiana Oncoematologia Pediatrica) LH 2004 protocol who relapsed or were primary refractory after first-line therapy received salvage treatment consisting of iphosphamide based regimen (IGEV) for 3/4 courses. Patients in complete remission (CR) PET/CT scan negative were submitted to APBSCT with BEAM conditioning regimen. PET/CT positive patients recieved a second line salvage treatment prior APBSCT. All patients received primary prophylaxis of febrile neutropenia with granulocyte *colony-stimulating* factor.

Table 1. Patient characteristics.

Patients	6 (n)	100 (%)
age yr, mean (range)		
at diagnosis	14.8 (11-17)	
at APBSCT	16.3 (12-18)	
Stage at diagnosis		
I-II	3	50
III-IV	3	50
AIEOP LH 2004 protocol risk group		
Gruop 1	0	0
Gruop 2	2	33
Group 3	4	66
Initial chemotherapy		
n. 4 COPP/ABV	4	33
n. 6 COPP/ABV	2	66
Status at salvage treatment		
Refractory	3	50
Relapsed	3	50

Results. From May 2008 to August 2011 six consecutive relapsed/primary refractory HL patients were treated. Clinical characteristics of patients are reported in Table 1. After IGEV regimen 4 patients achieved CR PET/CT negative (67%) and 2 patients were PET/CT positive (33%). The last 2 patients became PET/CT negative after second line salvage treatment consisting of *platinum-based* chemotherapy (ESHAP or DHAP). All patients received APBSCT. None of the patients experienced nonhematological toxicities. One patient, refractory to first line therapy, relapsed after 10 months from APBSCT and received BEACOPP salvage treatment followed by allogeneic bone marrow transplantation. All patients are alive and in CR PET/CT negative after a median follow-up of 11 months (range 5-45), so far. **Conclusions.** High dose chemotherapy followed by APBSCT has resulted in long-term disease-free survival in patients with refractory/relapsed HL. Response to pre-transplant salvage chemotherapy is the most important prognostic factor for outcome in this setting. IGEV is safe and effective salvage chemotherapy in adult HL patients, but there aren't data on pediatric safety and efficacy of this regimen. In our experience IGEV chemotherapy seems very effective in pediatric patients inducing high proportion of pre-transplant CR without relevant toxicities. Further investigations are warranted in a large cohort of patients in order to define the value of IGEV regimen in children affected by refractory/relapsed HL.

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THE IMPORTANCE OF PET/CT AS METHOD OF EVALUATION OF EARLY RESPONSE TO TREATMENT IN LH

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Background. The current standard treatment has increased the long-term survival from Hodgkin Lymphoma (HL) to more than 80% . However, the long-term adverse effects of treatment, such as secondary malignancies, cardiovascular and pulmonary complications, requires a risk-adapted lymphoma treatment with a early and accurate assessment of prognosis. **Aims.** The aim of this study is asses the importance of PET/CT as method of evaluation of early response to treatment in LH. **Methods.** Retrospective-prospective analytical study of a cohort of 61 patients diagnosed with Hodgkin Lymphoma at The Miguel Servet´s Hospital (Zaragoza, Spain), during the period 2002-2011. Treatment: Patients received first-line treatment based on the extent and manifestations of the disease, ABVD for stage I,II and IIIA and BEACOPP14 for stages IIIB and IV. Image study: All patients went under evaluation with PET/CT after 2 cycles of chemotherapy and at the end of 6 cycles of treatment. Hypermetabolic activity was considered positive when SUV > 2. 5. **Results.** Of 60 total patients, 58 were evaluated; 62 % (36) were men and 38 % (22) woman; the average age was 35. 2 ± 12. 9 years. For extra medical reasons no all patients were evaluated with PET/CT after 2 cycles of chemotherapy (total: 9). In 72. 4 % (42) of patients, early PET/CT performed after two cycles demonstrated non hypermetabolic disease activity. Of these 42 patients, 90. 4 % (38) were PET/CT negative at end of complete treatment with a progression free survival (PFS) at 24 months of 71 % vs 0 % for the group that had early PET/CT positive (P<0. 05); 47 patients had no disease activity as evidenced by CT/PET at end of treatment, in this group 80. 8 % (38) showed a localized stage at diagnosis; 11 patients had CT/PET positive at end of complete treatment, of this group 90. 9 % (10) showed an advanced stage at diagnosis. **Conclusions.** The PET/CT as a method for assessing the early response to LH treatment is useful, to be a strong predictor in PFS in HL. In cases of HL with localized stages at diagnosis and an interim CT/PET negative after two cycles of chemotherapy it could consider not doing a second study at end of treatment.

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RISK PROFILE OF RELAPSED/REFRACTORY HODGKIN LYMPHOMA PATIENTS TREATED WITH HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANT - A SINGLE CENTER EXPERIENCE

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Background. High dose therapy and autologous stem cell transplantat (SCT) has become the optimal therapeutic approach in patients with refractory and relapsed Hodgkin's lymphoma (HL). Historically, patients who relapsed after a full course of chemotherapy had a low chance for cure with second-line

treatment, with the duration of initial remission as significant predictor of subsequent response and relapse-free survival. There is intent to cure the disease in all stages, and long-term survival exceeds 85 percent for all stages. **Aims.** The aim of this study was to evaluate predictive value of clinical and laboratory parameters, as well the clinical course of relapsed and refractory patients with HL who were treated with high dose chemotherapy followed by autologous SCT. **Methods.** This retrospective study included 45 (25F/20M) patients - aged 30. 9 (20-46) years - with relapsed or refractory HL who were initially treated with ABVD therapy during the period 2005-2010. In the course of relapse or refractory disease, all patients received DHAP as salvage, and BEAM as conditioning regimen due to autologous SCT. **Results.** There were 13 patients in II CS defined as early unfavorable group, while 32 patients had advanced disease in III and IV CS. Treatment outcome on the initial ABVD therapy was as follows: CR in 19 (42. 2%), PR in 3 (6. 7%), SD in 11 (24. 4%), and PD in 12 (26. 7%) of patients. Among clinical characteristics bulky mediastinal mass was present in 60%, lymphadenopathy in 88. 8%, splenomegaly in 13. 3%, hepatomegaly in 8. 9%, and lung infiltration in 35. 6% of patients. Laboratory parameters demonstrated anaemia in 55. 6%, lekocytosis in 42. 2%, as well as lymphopenia and trombocytosis in 71. 1% patients. Low albumins were present in 71. 1%, and elevated ESR, CRP and beta2microglobulin in more than 90% of patients. Median duration of mobilzation (G-CSF 10µg/kgbm) was 6 days, CD34+ count was 8. 97 range 2-29 (x10E6/kgbm), median CD34+ cell viability was 79% (65-95%). Median duration of period for WBC engrftment was 13. 9 (9-19) days, and for PLT was 13. 1 (9-20) days. Deaths related to treatment toxicity were not recorded. Survival after auto SCT was 31 (1-45) months, and overall survival (OS) was 86 (12-144) months. Overall response rate at d+100 was 68. 8%, with 48% of CR. Survival analysis revealed that patients who achieved CR had significantly longer OS (p=0. 0001). Also, leukocytosis (p=0. 0001), lymphopenia (p=0. 026), trombocytosis (p=0. 0009), and anemia (p=0. 0185) had negative impact on OS. There was a strong trend toward worse OS in patients with bulky mediastinal tumor (p=0. 065). Using multivariate Cox regression analysis the most predictive prognostic factor for OS was treatment outcome at day +100. **Summary and Conclusions.** High-dose therapy and autologous SCT are adequate and safe therapeutic options for poor prognosis refractory/relapsed patients with HL, and posttransplant CR at day +100 could be the most predictive factor for long term survival.

Table 1.

Characteristics	No. of Patients	%
Total No. of patients	56	
Male/female	31/25	
Median age (range)	48.5 (16-65)	
Underlying disease		
Non-Hodgkin's lymphoma	18	32.1
Acute myeloid leukemia	17	30.4
Multiple myeloma	7	12.5
Myelodysplastic syndrome	5	8.9
Aplastic anemia	5	8.9
Acute lymphoblastic leukemia	3	5.4
Chronic myelogenous leukemia	1	1.8
Transplant type		
Autologous	24	44.7
Allogeneic	32	55.3
Conditining regimen		
Myeloabaltive	23	71.8
Non-myeloablative	9	28.2
Stem cell source		
Peripheral blood	53	94.6
Bone marrow	2	3.6
Cord blood	1	1.8
GVHD porphylaxis		
FK506 + Sirolimus	15	46.9
FK506 +MTX	14	43.8
CsA + MTX	3	9.3

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MICAFUNGIN AS PROPHYLAXIS OF INVASIVE FUNGAL INFECTION IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Invasive fungal infection (IFI) such as candidiasis and mold infections cause significant morbidity and mortality in hematopoietic stem cell transplantation (HSCT). Although prophylactic antifungal therapy with fluconazole has become the standard care for these patients, it has been associated with the emergence of fluconazole-resistant Candida infections. Additionally, fluconazole is not reliably effective against invasive aspergillosis. Micafungin is a antifungal agent of the Echinocandin class. This drug have potent antifungal activity against both Candida spp. and Aspergillus spp. Moreover, micafungin is also effective against Fluconazole-resistant Candida albicans. Between January and December 2010, we conducted a retrospective study to evaluate the usefulness of the administration of micafungin (Mycamine®) as a prophylactic antifungal therapy for patients undergoing HSCT. The primary objective was to evaluate the treatment success, which was defined as the absence of proven, or probable IFI through the end of therapy and as the absence of proven or probable IFI through the end of the 4-week period after treatment . The secondary objective was incidence of micafungin-related toxicity. Adult patients with hematological and non-hematological malignancy undergoing auto & allo-HSCT were eligible for the study. Micafungin was started at a daily dose of 50 mg once a day intravenously over 1 hour from day 1 after HSCT. Therapy was continued until 3 days after hematological engraftment (defined as an absolute neutrophil count of over 500/uL after the nadir). Fifty-six patients were evaluated in this study. The median durations of administration of micafungin were 18 days (range 10-45 days). Prophylactic success was achieved in 55 patients (98. 2% of the 56 evaluable patients). One patient were diagnosed as having probable invasive pulmonary aspergillosis. Micafungin was well tolerated with no evidence of significant toxic effects in all cases. Prophylactic micafungin (50mg/day) is effective and safe in patients undergoing HSCT. Mild to moderate hepatic impairment does not warrant changes in dose. A multicenter, randomized study to determine the appropriate effective Dose of micafungin is needed.

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EVALUATE THE LABORATORY AND RADIOLOGICAL EXAMINATION FOR PREDICTING OUTCOME AND ADJUSTING EMPIRICAL ANTIFUNGAL THERAPY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A MULTICENTER, RETROSPECTIVE STUDY

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Background. Due to the difficulty of early diagnosis of invasive fungal diseases (IFDs), empirical antifungal therapy has been the standard practice for immunosuppressed patients with persistent fever after receiving broad-spectrum antibiotics, although more than a half of antifungal agents may be overused. In addition, there is no globally-recognized course of empirical treatment, which may result in continuing use of extra antifungal agents for a long period. **Aims.** In our retrospective multicenter study, we evaluate the laboratory and radiological evidence for predicting higher response rate of empirical antifungal therapy. Meanwhile, the proper duration of empirical treatment would be explored. **Methods.** From fifteen medical centers in China, 376 patients with hematological malignancies were enrolled. When persistent fever (T>37. 5ºC, >24hrs) was developed in patients, a diagnostic work-up was performed to identify the reason of fever, including culture of blood or samples from suspected infected sites, serum GM detection, radiological examination, etc. . Evidence of IFDs referred to any positive results of the above tests. As the initiation of antifungal therapy was determined by physicians, all patients were divided into three groups: group A contained patients who received preemptive antifungal therapy, group B contained patients who received empirical antifungal therapy and acquired evidence of IFDs within two weeks after initiation of antifungal treatment, and group C contained other patients who also received empirical therapy but were not included in group B. The duration of antifungal therapy and the agents were also decided by physicians in accordance with the guidelines. And the results of treatment were evaluated as response or failure according to the patients' signs and symptoms and the improvement of imaging lesions. **Results.** 87, 105 and 184 patients were allocated to group A, B and C respectively. The efficacy of treatment in group A was higher than that in groups B+C (59. 8% vs. 34. 9%, *P*<0. 001). And the efficacy in group B was significantly higher than that in group B (63. 8% vs. 18. 5%, *P*<0. 001). Among patients after intensive chemotherapy, the efficacy of preemptive strategy was higher than that in empirical strategy (64. 8% vs. 32. 0%, *P*<0. 001),

while they were comparatively close in patients undergoing HSCT (51. 5% vs. 39. 2%, *P*=0. 203). The median time from the onset of fever to initiation of antifungal therapy in groups A, B and C were 5, 4 and 4 days respectively, and the mean start time among patients who responded to antifungal treatment was not earlier than that in patients with failure outcome (4. 5±1. 72 vs. 4. 6±1. 97, *P*=0. 505). In group C, the low incidence of IFDs (one probable and two possible IFDs) and low efficacy of empirical antifungal therapy may suggest that more than two weeks of empirical antifungal therapy would not further benefit these patients. **Conclusions.** Positive evidence of IFDs could predict the higher response rate of empirical antifungal therapy. And the preemptive strategy may be more suitable for patients with relatively low incidence of IFDs, while for patients with high risks of IFDs the empirical antifungal therapy should be emphasized.

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MICONAZOLE MUCOADHESIVE BUCCAL TABLET IN PREVENTION OF HIGH DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION (HDT/ASCT)-INDUCED MUCOSITIS - A PILOT STUDY

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Background. Mucositis is a major cause of morbidity in HDT/ASCT patients, especially responsible for pain, dysphagia, malnutrition and potential infectious portal of entry. Microbial colonization has a great implication in the mucositis pathogenetic pathway and so, yeast prevention with oral amphotericin B suspensions are usually given to these patients even tough there is no clear consensus. Miconazole mucoadhesive buccal tablet, a continuously released and well-tolerated anti-mycosis has shown efficiency in oral yeast treatment and could be an alternative to oral amphotericin B suspensions, whose tolerance can be difficult. **Aims.** To analyze tolerance and efficiency of miconazole mucoadhesive buccal tablet in mucositis prevention, in substitute to oral amphotericin B suspensions, the current reference treatment in our center. **Methods.** We compared 60 patients from whom informed consent was obtained and who received daily miconazole mucoadhesive buccal tablet from November 2008 to August 2011 with 44 control patients who received oral amphotericin B suspensions three times a day (one gargled dose and one swallowed dose) from January 2006 to the implementation of the study, treated in our center with HDT/ASCT. These patients were treated for either acute myeloid leukaemia with busulphan-melphalan, lymphoma with a BEAM conditioning regimen or for myeloma with high-dose melphalan. All patients received simultaneously chlorhexidine and bicarbonate based mouthwash. Neutropenia prevention usually consisted of filgrastim infusions for control patients and pegfilgrastim infusions for miconazole patients. No patient received antibiotic prophylaxis.

Table 1.

	Control group (n=44)	Miconazole group (n=60)	p
Age	49,11	51,28	0,2
Female patients	45%	43%	0,81
Acute myeloid leukemia	6,82%	3,33%	
Myeloma	38,64%	56,67%	
Lymphoma	54,55%	40,00%	0,17
Total hospital stay (days)	23,36	22,3	0,13
Hospital stay after engraftment (days)	16,36	15,3	0,09
Total CD34 (10^6/kg)	5,16	5,1	0,45
Neutropenia <500/mm3 (days)	8,07	7,42	0,13
Leucopenia <1000/mm3 (days)	8,11	7,77	0,28
Nefopam (days)	5,73	5,4	0,37
Nefopam doses	389	437	0,31
Morphine (days)	4,93	3,85	0,12
Morphine doses	125	121	0,44
Parenteral nutrition (days)	9,95	8,98	0,15
IV Antibiotics (days)	12,34	7,8	<0,0001
Antiviral (days)	10,5	16	<0,0001
IV Antifungal (days)	3,55	1,41	0,02
Yeast stool contamination	45%	37%	0,36

Results. The patients' characteristics were similar in both groups except a trend to more lymphoma patients in the control group. Miconazole patients tended to have less morphine use, shorter morphine use and shorter parenteral nutrition use in favour of less severe mucositis. Also, there was shorter antibiotic use which can be accredited to fewer infections in the miconazole group secondary to mucositis prevention with the acknowledgment that local practice changes can be considered as confusing factors (pegfilgrastim use, antiviral prophylaxis started on admission date in miconazole patients). Fewer intravenous antifungal treatment, less yeast stool contamination and no candidemia occurrence support that miconazole is a good fungal control option. The reduction of all of these parameters is responsible for a shorter hospital stay in the miconazole group. After sub-group analysis, these results were especially confirmed in myeloma patients with shorter hospital stay (14. 5 versus 16. 6 days after engraftment; p=0. 004), shorter morphine use (1. 82 versus 3. 59 days; p=0. 08), shorter parenteral nutrition use (7. 74 versus 8. 05 days; p=0. 38) and shorter antibiotic use (4. 29 versus 10. 59 days; p<0,0001) with similar mean neutropenia (<500/mm3) duration (5. 65 versus 5. 35 days; p=0. 19) whereas there was no improvement on any parameters in lymphoma patients. Six patients discontinued miconazole, and were not included in the analysis, three due to the impossibility of obtaining stable buccal adhesion, and three following trial withdrawal due to the investigator's discretion. **Conclusions.** Miconazole mucoadhesive buccal tablets, well tolerated in our patients, can be a cost-equivalent alternative to oral amphotericin B suspensions in preventing HDT/ASCT-induced mucositis with less intravenous antifungal use, especially in myeloma patients receiving high-dose melphalan conditioning regimen.

1421

PREVENTING INVASIVE FUNGAL INFECTIONS (IFI) USING ANTI-MOLD AZOLE ANTIFUNGALS IN PATIENTS RECEIVING HYPERCVAD

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Background. Fluconazole has long been the agent of choice for fungal prophylaxis in patients receiving intensive chemotherapy for hematologic malignancies, such as HyperCVAD. Invasive fungal infections secondary to mold are becoming an increasing issue in this population. Newer agents such as posaconazole and voriconazole have shown superior activity against mold in patients undergoing stem cell transplantation or induction chemotherapy for acute myeloid leukemia and are promising options in preventing IFI. **Aims.** 1) Determine incidence of IFI during HyperCVAD; 2) Evaluate efficacy of different antifungal agents fluconazole, posaconazole, and voriconazole in preventing IFI. **Methods.** A retrospective analysis of patients treated with HyperCVAD at our center between October 2006 and December 2011 was performed under an IRB approved protocol. Patients were evaluated until death, relapse, transplant, or completion of HyperCVAD therapy. Rituximab was given in patients who had CD20+ disease. Patients were given fungal prophylaxis with fluconazole, posaconazole or voriconazole. Due to drug interactions with vincristine, patients were instructed to hold their antifungal starting 24 hours prior to vincristine, restarting antifungal therapy 24 hours after administration of vincristine. **Results.** We identified and evaluated thirty-three patients. Median age was 57(range: 19-75), 64% ALL, 18% MCL, and 18% were other hematologic malignancies (CLL, MM, BL, DLCL, PTCL). Patients received a total of 146 cycles (median 5). Nine (27%) patients received fluconazole prophylaxis, while 24 (63%) received either voriconazole or posaconazole prophylaxis. Overall incidence of proven IFI was 6% (2/33), occurring only in the group who received fluconazole as prophylaxis (22%, p=0. 068). One case of IFI was speciated as *Aureobasidium pullulans* (patient alive after surgery, liposomal Amphotericin B and Allogeneic transplant), and the second case was *Fusarium* (patient died). There were no cases of resistant IFIs in patients receiving posaconazole or voriconazole. Increases in LFTs occurred in the 11% of patients, but were transient and related to treatment with chemotherapy. No patients developed grade 3/4 elevations in hepatic function. Drug interactions between azole antifungals and vincristine are a major concern which can lead to serious complications and delays in therapy. The common practice at our institution is to hold azole antifungals 24 hours prior to, during and 24 hours after vincristine is administered. Of our patients, 25/33 were evaluated for neuropathy. Nine of 25 (36%) patients developed grade 1/2 neuropathy. No patient developed grade 3 or higher neuropathy. **Summary and Conclusions.** The overall rate of IFI at our institution is lower than previously reported in the literature in groups using fluconazole alone as prophylaxis. The rate of breakthrough IFI in patients receiving antifungal prophylaxis was higher in the group using fluconazole (22% vs. 0%). This data shows IFIs are successfully prevented in patients receiving HyperCVAD by using broad spectrum antifungal agents.

1422

A TEAM-BASED, MULTIDISCIPLINARY APPROACH TO REDUCING PERIPHERALLY-INSERTED CENTRAL CATHETERS (PICC) COMPLICATIONS IN HAEMATOLOGICAL PATIENTS:A PROSPECTIVE STUDY

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Introduction. Few data is available on clinicalefficiencyand safety of Peripherally-Inserted Central Catheters (PICC)in haematological patients (HP). Preliminary

data suggests a highrate ofPICC-relatedcomplicationsinthisgroup ofpatients. To highlightthe role of PICC in HP receivingintensive chemotherapy,a prospective studyof PICCwas conducted in our Unitfrom November 2010 to February 2012 to determine theincidence of PICC-relatedcomplicationsafter evidence-based interventions. **Methods.** Amultidisciplinary program was implemented involving healthcare professionals who insert and maintain PICCs:1) maximum sterile precautions during catheter insertion and aftercare;2)chlorhexidine preparation for skin antisepsis; 3) confirmed PICC tip placement by chest radiography; 4) information from weekly PICC line review;5) health-care managers allocating resources, and 6) patients capable of assisting (i. e. patient education regarding provision of their own catheter care). **Results.** Twenty-seven consecutive 5-Frenchpolyurethane double lumen PICC (Bard Access Systems, Salt Lake City, Utah, USA)were insertedin 20patients [M/F 13/7, median age 46. 25 years, range 11-77] for a total of 3750 days (average 138. 88, range 4-420+). Five patients had ALL, 5 AML, 6 aggressive NHL, 1 MDS/MPD, 2 MM; as to disease phase, 13 PICCs were atonset, 7 in complete response (5 consolidation and 2 intensify phase), and 7 at disease relapse. PICCs were successfully inserted in all cases (most of them in via cephalic vein). At insertion,platelets count was < 50 x 10^9/l in 6/27 cases (22. 2%), andWBC count was < 1. 0 x 10^9/l in 2/27 cases (7. 41%). Overall thirty PICC-relatedcomplications developed in 70. 37%of the catheters (5. 06 events/1000 catheter days). In order, frequency complications were: a) 20 (66. 6%) mechanical complications (5. 33/1000 catheter days), b) 9 (30%) PICC-related infections (2. 4/1000 catheter days), and c) 1 (3. 3%) symptomaticPICC-related thrombosis (0. 27/1000 catheter days);[see Table 1 for details]. Twenty-one PICCs were removed after an average period of 126 days (range 4-232); the reasons were: completion of treatment in 9 patients, death unrelatedto PICC in 3, voluntary retirement in 1 and catheter-related complications in 8 (3for local infection, 1 for thrombosis and 4 for accidental extraction). Six PICCs (48. 5%) are still in use for an averageperiod of 147,16 days (range16 - 295). Our PICC complications rate appears favourably with published series on non-tunneled and noncuffed central venouscatheters (CVC-related symptomaticthrombosis up to 25% and CVC-related infections 4. 68-6. 61events/1000 catheter days)in the haematological setting. **Conclusions.** Our data indicates that a multidisciplinarycompetence program to PICC seemsto be a useful, safe and promising alternative to conventionalCVC for haematological patients receiving intensive chemotherapy.

Table 1.

Complications	Number (%)	Per 1000 CVC days
PICCs related infections:	9 (30%)	2.4
- Definite CRBSI	0	0
- Local infections	2 (6.6%)	0.53
- Exit site infection	7 (23.3%)	1.8
PICCs related thromboses:	1 (3.3%)	0.27
- Symptomatic TVP	1 (3.3%)	0.27
PICCs mechanical complications:	20 (66.6%)	5.33
-Obstructions	16 (53.3%)	4.26
- Accidental dislodgement	4 (13.3%)	1.06

1423

EVALUATION OF BONE MARROW EXAMINATION IN PATIENTS WITH HEPATITIS C VIRUS INFECTION

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Background. Over 170 million individuals are infected with Hepatitis **C** virus worldwide **.** Hepatitis **C** prevalence is higher in some countries in Africa and Asia. The report published by the Egyptian Demographic Health Survey [EDHS] stated that in Egypt there is an overall Anti-HCV antibody prevalence of 14. 7% [estimated to be about 12 million individuals] and the number of Egyptians estimated to be chronically infected was 9. 8% [about 8 million individuals] . Several extrahepatic manifestations have been reported in the natural history of hepatitis C virus infection. Up to 40-74% of patients infected with HCV might develop at least one extrahepatic manifestation during the course of their disease**.** Hematological manifestations are among the commonest extrahepatic manifestations of HCV infection. Patients with HCV infection can develop peripheral blood cell count abnormalities that are commonly attributed to hypersplenism, antiviral therapy, decreased thrombopoietin levels, and/or autoimmune mechanisms **.** Mixed cryoglobulinemias and lymphoproliferative diseases have an increased incidence with chronic hepatitis C. **Aims.** Evaluation of bone marrow findings in patients infected with hepatitis C virus presenting with peripheral blood cytopenias. **Methods.** This study was conducted on 35 patients with chronic hepatitis C virus infection presenting with monocytopenia,bicytopenia or pancytopenia. Patients were subjected to the following: history taking, physical examination and laboratory investigations including:Complete blood picture, Erythrocyte Sedimentation Rate(ESR) Lactate Dehydrogenase enzyme(LDH). Hepatitis C markers (Anti-HCV Ab using third generation ELISA technique) HCV RNA: using real -time PCR when possible. Bone marrow aspiration for morphological examination for all patients. Bone marrow trephine biopsy for histopathological examination when indicated. Overall BM cellularity, cellularity of myeloid, erythroid and megakaryocytes series. and BM infiltration, fibrosis, dysplasia and iron stores were assessed. Flowcytometry and cytogenetics in selected patients. Patients under interferon therapy, or with hepatocellular carcinoma were excluded from the study. **Results.** The spectrum of hematological findings among recruited patients was as follows11 cases (31. 44%) suffered from B-Non Hodgkins Lymphoma, 4 cases (11. 43%) suffered from Immune thrombocytoprnia, 1case(2. 86%) suffered from anemia of chronic disease, 1 case(2. 86%) suffered from aplastic anemia, 2 cases (5. 71%) suffered from Auto Immune Hemolytic Anemia, 3 cases (8. 57%) suffered from acute myeloid leukemias, 3 cases (8. 57%) suffered from acute lymphoblastic leukemia, 1 case (2. 86%) with chronic lymphocytic leukemia, 3 cases (8. 57%) with hypercellular bone marrow secondary to hypersplenism, 1 case (2. 86%) suffered from myelofibrosis, 3 cases (8. 57%) found to be MDS, 1 case (2. 86%) found to be Evan's syndrome and 1 case (2. 86%) with sideroblastic anemia. **Conclusions.** There was a wide variety of hematological diseases in patients with hepatitis C with peripheral cytopenias. B-NHL is the most frequently detected problem among recruited patients(31%) There were significant data involving relation of bone marrow dysplasia and fibrosis to age of patients

1424

COMPLIANCE WITH FEBRILE NEUTROPENIA MANAGEMENT AN EXPERIENCE FROM QATAR

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Background. Febrile neutropenia (FN) is associated with significant morbidity and mortality. Managing infections in neutropenic patients remains a dynamic process, making necessary timely and efficient empirical antibiotic therapy. The implementation of critical pathways has been suggested as a strategy to improve clinical effectiveness implementation of a critical pathway seems to be an effective strategy to improve clinical outcomes in patients hospitalized with FN. **Aims.** This study evaluated the compliance with an institutional critical pathway for the management of FN and the impact on clinical outcomes at Al-Amal cancer centre in Qatar from January 2007 to December 2011 and presented with FN (186 episodes). **Patients and Methods.** We performed a cohort study that retrospectively included patients hospitalized from January 2009 to December 2011 (186 episodes). FN protocol for management of high risk patients consist of initial assessment on day 0 (History, physical exam, CBC, U&E, blood C/S, one from each lumens of the central line and one peripheral, coagulation profile, CRP, midstream urine culture, sputum for M/C/S, feces for M/C/S, swab for wound all wounds, CX-ray, taping fluid if any. Initial Antibiotics consists of Amikacin & Tazocin, and Vancomycin is considered in particular situations. Reassessment on day 3 if the patient remains a febrile, and if etiology identified to adjust to most appropriate treatment, continue antibiotic to complete the course of therapy, monitor resolution of infection, discontinue antibiotic if ANC > 0. 5. And if there is no etiology identified: continue same antibiotics, if clinically stable and no evidence of infection discontinue the antibiotics if ANC > 0. 5. If febrile, add vancomycin and consider early introduction of liposomal amphotherecin B, if patient is blastic or has prolonged neutropenia. A reassessment on day 5 continue same antibiotic until ANC > 0. 5 and stop antimicrobial with in 48 hours if the patient is afebrile. If patient remains febrile, monitor ANC and if its < 0. 5, blood culture negative, workup possible fungal infection, add liposomal amphotherecin B. **Results.** before the critical pathway was introduced. This study showed low rate of full compliance 142 episodes (76%) versus 44 episodes (24%) of violations after critical pathway implementation. The major violation of the protocol occurs mainly in ER. **Conclusions.** Despite the moderate adherence observed, we recorded decrease in-hospital all-cause mortality in the sample studied after protocol implementation. Implementation of a critical pathway seems to be an effective strategy to improve clinical outcomes in patients hospitalized with FN.

1425

IMPACT OF BLOOD STREAM INFECTIONS AND PSEUDOMONAS AERUGINOSA IN A HAEMATOLOGICAL DEPARTMENT: THE EXPERIENCE OF 3 YEARS

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Background. Mortality rate of bloodstream infections (BSI) among neutropenic haematological patients ranges between 5-10%. Multi-drug-resistant pathogen has been associated with increased mortality. **Aims.** To determine the impact of

Pseudomonas Aeruginosa (Pa) on the outcome of haematological patients in our Institution, particularly Multi-Drug-Resistant Pa (MDRPa) and Extensive-Drug-Resistant Pa (EDRPa). **Materials and Methods.** From January 2008 and February 2011 we followed up 220 patients (pts) treated with high-dose chemotherapy and hospitalized at S. Gerardo Hospital,Monza,Italy. All strains were isolated in Haematological Department. Haematological disorders were: 159 acute myeloid leukemia (72%), 48 lymphoproliferative diseases (22%) and 13 others. Pts under 65 years old were 156/220 (71%). MDRPa and EDRPa are defined as literature reported (Falagas, Clinical Infectious Disease, 2008). **Results.** We have found 217 BSI from 220 pts and 29 Pa isolated from 31 pts. Mortality rata in BSI was 21/193 sepsis (10. 8%). 24 BSI were presented as a septic shock and in this group 15 pts were died (62. 5%). All pts were neutropenic and didn't receive any antibactirial prophylaxis. BSI-etiology and related mortality rate were: Candida 7 (1 death: 14. 3%), Clostridium 6 (0), Corynebacterium 7 (0), Enterobacter 13 (1 death: 7. 7%), Enterococci 32 (8 deaths: 25%), Escherichia Coli 59 (4 deaths: 6. 8%), Klebsiella 4 (2 deaths: 50%), Coagulase negative Staphilococci 45 (6 deaths: 13. 3%), Staphilococcus Aureus 5 (1 death: 20%), Stenotrophomonas 10 (4 deaths: 40%). In picture 1 is shown the relationship between etiology-incidence and its mortality: some pathogens were less frequent but they were related with a high mortality rate (Klebsiella, Enterococcus Faecium). On the other side, Escherichia Coli was the most frequent bug with the less mortality: deaths occurred in pts with relapse leukemia and poor prognostic status (no one was ESBL positive). Deaths related to Pa were 9/29 (31%) but splitting different Pa with the resistance profile, MDRPa had a mortality rate of 1/1 (100%) and EDRPa of 7/7(100%). All non MDRPa responded to first line therapy except 1 pt who died for multiorgan failure. Other Pa were resistant to fluoroquinolones and third generation cephalosporine. All EDRPa showed a resistance in carbapenemics and positive metallobetalattamasis, in all cases colimicin was the only active antibiotic. Both state of disease and number of previous hospitalization were not correlated with a different profile of resistance. The lack of temporal and physical proximity and the strains' genetic analysis had excluded the presence of a EDRPa or Pa out-break. **Summary and Conclusions.** Mortality data on Pa in our series are worsen than what is reported in literature (40% mortality for MDRPa in Trecarichi, Haematologica, 2011). Even though the numbers are small, the impact on survival is relevant (100% mortality for MDRPa-EDRPa). Since now, no active combination of antimicrobial therapy was effective to EDRPa. The emergence of MDRPa and EDRPa in our haematological pts is a new challenge for clinicians. This work wants to be the base of an epidemiology surveillance in order to understand if there is any better approach to infection in high-risk haematological pts.

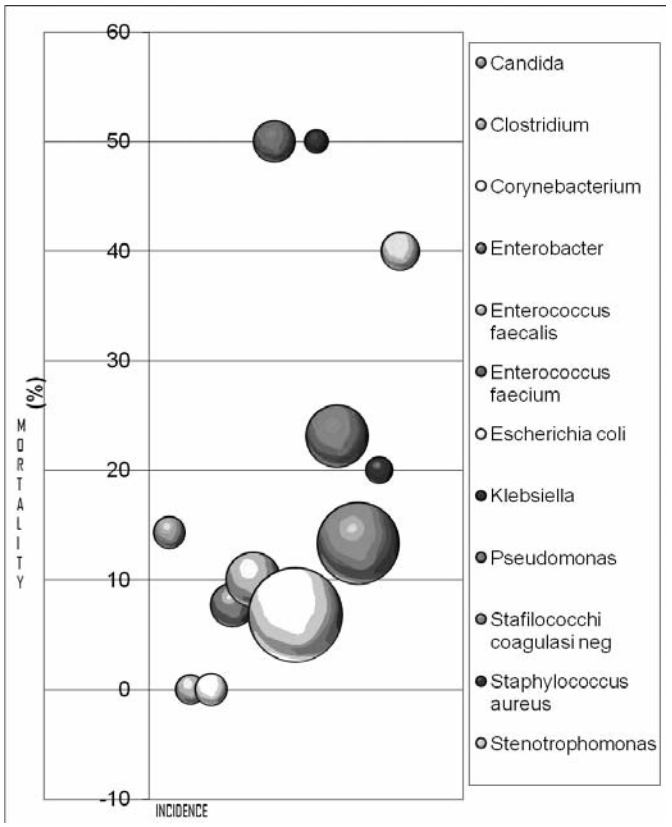

Figure 1.

1426

INFECTION DUE TO STAPHYLOCOCCUS AUREUS IN PATIENTS WITH HEMATOLOGICAL DISEASES: PROGNOSTIC FACTORS OF BACTEREMIA AND SEVERE OUTCOME

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Background. Staphylococcus aureus is a clinically significant pathogen, usually associated in causing high morbidity nosocomial infections in cancer patients. The aim of this study was to determine the risk factors associated with bacteremia and severe outcome in patients diagnosed with hematologic diseases and Staphylococcus aureus infections. **Results.** A total of 85 Staphylococcus aureus isolates were collected from 70 patients aged 2-81 years: 47 with acute leukemia (67. 1%), 14 with lymphoma (20 %), and 9 with other hematologic disorders (12. 9%). The median age of the patients was 27 years. M/F was 1. 9 (P=0. 0006). Isolates were collected mostly from bloodstream (43. 5%) and skin lesions (48. 2%). 32. 9% and 33. 3% of the isolates were resistant to oxacillin and amikacin respectively. 2 patients(2. 8%) developed Severe sepsis/septic shock and 2 patients (2. 8%) developed respiratory failure . Crude mortality due to septic shock and pneumonia occurred in 2 patients (2. 8%). Factors associated with risk of bacteremia due to Staphylococcus aureus were: WBC < 2 x 10^9/l (P=0. 01,OR 3. 5;95%CI: 1. 3-9. 6), absolute neutrophil count (ANC) <1. 5x10^9/l (P=0. 001;OR 9;95%CI:2. 2-35. 4),Calcium level <2. 1mmol/l (P=0. 022,OR 4. 2;95%CI:1. 1-15. 3),low alkaline phosphatase level <80 (P=0. 036,OR 3. 6;95%CI:1-12. 1), glycemia > 6. 4 mmol/l (P=0. 01,OR 4. 8;95%CI:1. 4-16. 3) and female gender (P=0. 037,OR 2. 6;95% CI:1-6. 8). Factors associated with severe complication and death were: Neutropenia (P=0. 03), low Hemoglobin level (P=0. 011), low platelet count (P=0. 015), high CRP level (P=0. 04) and high Procalcitonin level (P=0. 016). **Conclusions.** This study revealed that CRP and Procalcitonin level are markers of severity in patients with hematological diseases and infection due to Staphylococcus aureus.

1427

UTILITY OF 18F-FLUORINE DEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY IN GUIDING ANTIFUNGAL THERAPY IN A PATIENT WITH DEEP-SEATED MUCORMYCOSIS

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Background. Mucormycosis is a rapidly-progressive deep fungal infection primarily affecting patients with underlying metabolic or immunological compromise. The management of patients suffering mucormycosis is very challenging. **Aims.** This study aims to assess the roles of 18F- fluorine deoxyglucose positron emission tomography/computed tomography (FDG PET/CT) in the management of patients with deep-seated mucormycosis. **Methods.** A series of FDG PET/CT images and consequential changes of the treatment plan of a 23-year-old patient who suffered aplastic anemia and rhinocerebral mucormycosis were analyzed. **Results.** PET/CT demonstrated the lesion was 18F-FDG avid and detected some additional positive lymph nodes in both necks. The findings of the first PET/CT scan directed accurate biopsy and guided the initial therapy plan. The judgment of the follow-up PET/CT imaging leaded to the adjustment of antifungal regimen, and then the conclusion from the final PET/CT study resulted in the successful termination of the long-term antifungal therapy. **Conclusions.** FDG PET/CT appears to be a valuable modality for the diagnosis and guiding the antifungal therapy against deep-seated mucormycosis.

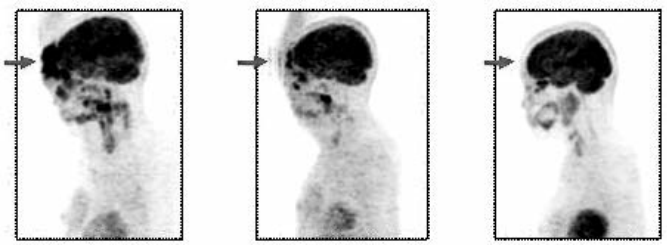

Figure 1.

1428

SAFETY AND EFFICACY OF ANIDULAFUNGIN TREATMENT IN PATIENTS WITH HAEMATOLOGICAL DISEASE

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Background. Anidulafungin is a new echinocandin with broad-spectrum activity against *Candida* and *Aspergillus* species. It does not require dose adjustment in renal or hepatic insufficiency and shows few drug interactions. All of these characteristics are attractive for its use in haematological patients, however little experience has been reported. **Aims.** To analyze efficacy and safety of anidulafungin treatment in patients with haematological disease. **Methods.** Between September 2011 and January 2012, 16 patients (8 male, median age: 60 years (46-88) with haematological diseases (Acute leukaemia 4; MDS 3; NHL/CLL 4; Myeloma 2; Aplastic Anemia 1; chronic MPN 2) received anidulafungin for at least 5 days in our centre. Therapy was given as empirical treatment in neutropenic fever (5 chemotherapy, 5 allogeneic SCT and 1 aplastic anemia), as primary antifungal prophylaxis in non neutropenic high risk allogeneic patients (4) or as combined treatment of proven pulmonary aspergillosis (1). No previous history of invasive mold infection was reported. **Results.** Anidulafungin was given for a median of 9 days (5-53) and no patient developed breakthrough fungal infection. In all subgroups of patients, liver function tests (AST, ALT and GGT) were significantly lower ($p<0,05$) following therapy and we did not see statistical differences in bilirubin and creatinine levels. Median days of an absolute neutrophil count below 100/mm3 and 500/mm3 in patients who received empirical therapy were 13 (7-18) and 16 (10-22) respectively. In the allogeneic group, calcineurin inhibitor levels were similar before and after treatment and those patients who received primary prophylaxis (all included in allogeneic group) were severely immunocompromised because of high dose corticosteroid treatment (3) or anti-TNF therapy for refractory acute gut and liver GVHD (1). Combined use of anidulafungin and voriconazol was given to a patient with allogeneic SCT and severe chronic GVHD who developed proven pulmonary aspergillosis and respiratory insufficiency, with successful evolution. At this time, 6 patients died because of GVHD (1) or disease progression (5). **Conclusions.** In our experience, anidulafungin shows an excellent profile of efficacy and safety in haematological patients but this needs to be confirmed in longer studies.

1429

CLINICAL FEATURES, DIAGNOSIS AND MANAGEMENT OF HEMATOLOGIC PATIENTS WITH BACTEREMIA IN A GENERAL HOSPITAL

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Background. Patients with hematologic malignancies who receiving chemotherapy develop severe neutropenia with negative impact on the evolution of infectious complications. Despite prophylactic measures, such as reverse protective isolation, HEPA filter, use of antibiotics with wide antibacterial spectrum, and use of stimulating factors hematopoietic growth cells, cases of sepsis causes high mortality and morbidity, therefore it is very important to optimize the management, diagnosis and treatment in this special situations. **Aims.** Evaluated cases of bacteremia and its management in hematological patients in relation to epidemiological characteristics, clinical data and infectious parameters. **Methods.** Retrospective study from January 2009 until December 2011. The starting point was all blood culture were ordered at this period, evaluate retrospectively epidemiological, clinical, and bacteriological characteristics and pharmacological treatment for those who were positive. **Results.** The total blood cultures requested in the period of 36 months were 780, of which [15%;n=115] were positive. In relation with the acquisition zone of blood cultures, 72%were obtained from peripheral access, 18% from central venous and 10% from subcutaneous reservoir. Positive blood cultures were in 49 patients, with an annual distribution of 18 patients in 2009, 13 in 2010 and 18 in 2011. The median age was 60. 8 years (range 20-81), with a predominance of males [n = 27 (55%)]. The pathologies was: 32 patients had myeloblastic leukemia, 9 patients with aggressive Non-Hogdkin ['Diffuse Large Cell (n = 8) and NHL' Mantle Cell '(n = 1)]. 5patients with Myelodysplastic Syndrome, and 3 patients with Multiple Myeloma. In relationship with active treated**:** 40patients (82%) had Active chemotherapy versus 9 patients (18%) who were of-treatment phase active. Figure 1. At the time of bacteremia, [63%;n = 31], had an absolute neutrophil count (ANC) less than 0. $5x10^9$/L [31%;n=15] ANC between 0. 5-1. $5x10^9$/L and [6%;n=3] had an ANC greater than 1. $5x10^9$/L. The bacteriological Results. [53%;n = 26] corresponded to Gram-positive cocci, [43%,n = 21] were Gram-negative bacilli and [4%;n=2] were fungi. Figure 2. The antibiotics used was:b-lactams (55%),glycopeptides (25%), quinolones (5%), nitroimidazoles (6%), antifungals (6%) and aminoglycosides (3%). 78% of patients have been dying due to the evolution of the disease, although none of them, the direct cause of death was immediate consequence of the infection. **Conclusions.** The active chemotherapy and the RAN low (less than 0. $5x10^9$/L) have shown in our series, as two clear risk factors for the development of sepsis in hematologic patients. By contrast, there are no large differences in tumor pathology base or chemotherapy protocol used. The type of germ has not significantly influenced the clinical course of patients in our study, although a high percentage of all positive blood cultures were from peripheral venous access extractions. No differences were found in relation to epidemiological factors (sex / age). Respect antibiotic treatment chosen, the use of broad spectrum antibiotics and the combination of them, and especially an early administration, have a very favorable influence on the resolution of the infection, with excellent data in our series. These data will be update in congress.

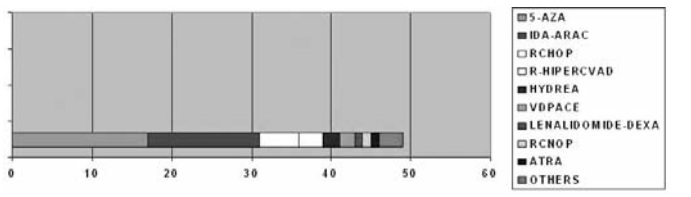

Figure 1. Chemotherapy treatments.

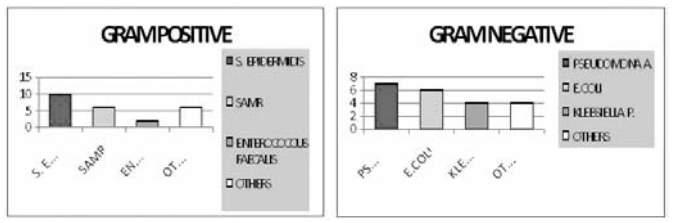

Figure 2. Types of germs in positive blood cultures.

1430

INVASIVE FUNGAL INFECTIONS IN HEMATOLOGIC MALIGNANCIES

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Background. Invasive fungal infections (IFI) are very frequent and serious complications after aggressive myeloablative regimens in patients (pts) with hematologic malignancies. **Aims.** To define possible prognostic factors for IFI in pts with hematologic malignancies. **Methods.** The data of 72 pts (m/f:41/31; Me age 53, range 20-76) . The majority of pts received prophylaxis for IFI: itraconazole 41 pts (57,1%) and fluconazole 19(26,2%). Diagnosis of IFI was performed according to EORTC criteria for possible,probable and proven infection, using mostly standard diagnostic procedures: blood cultures, serologic tests (galactomannan,anti Aspergillus IgM and candida-mannan), histopathological findings,X-ray, ultra sound and MSCT diagnostics. **Results.** The majority of pts 55(76,4%) had acute myeloid leukemia (AML) while other hematologic malignancies were rarer: acute lymphoblastic leukemia 6 pts,aplastic anemia 4 pts, Hodgkin¢s disease 2 pts, non-Hodgkin disease 2pts, chronic lymphocytic leukemia 2 pts and hairy cell leukemia 1 pt . IFI was diagnosed after induction therapy (th) in 40 pts(55,6%), "salvage" th in 18 pts (25%) and myeloablative th in remission in 14 pts (19,4%). There were 16 pts with invasive candidiasis (IC) (proven 13 pts, possible 3 pts),52 pts with invasive pulmonary aspergillosis (IPA) (proven 2pts, probable 20, possible 30 pts) and 2pts with proven invasive cryptococcosis and trychosporonosis, 2pts with zygomicosis. All pts had neutropenia with Me duration of 18 d (range:5-38), without significant differencies between IC and IPA . Pts with IC had more mucositis grade III and IV than pts with IPA, as well as pleural effusions ($p < 0,05$). Galactomannan test was twice positive in 20/52 pts(38,5 %), anti Aspergillus At IgM where positive at 13/20 (65 %) pts with probable and 12/30 (40%) pts with possible IA ($p>0,05$). Candida-mannan was positive once in proven IC pt. Pts with IC had Me survival of 4 wks, with mortality in 9/16 pts,while pts with IPA had Me survival of 16 wks and mortality in 31/52 pts. Me survival of other pts with IFI (1,5 wk) was more shorter vs pts with IC and

IPA (p < 0,05). All pts with fatal IFI had longer duration of neutropenia than other pts with IFI(Me 19,5 vs 15,5 d; p<0,05). Pts without any fungal prophylaxis had more deaths vs. pts with prophylaxis (p<0,05), as well as pts with IFI during induction or salvage th vs. pts receiving th in remission (p<0,05). **Conclusions.** The most significant risk factors for IFI in hematologic malignacies are: AML as a type of disease, myeloablative regimens used in induction and salvage th, prolonged period of neutropenia. IPA is the most prevalent form of IFI. IC should be considered in neutropenic febrile pts with pleural effusions and mucositis of high grade. Survival is shorter and the rate of deaths is bigger in pts with IFI during prolonged neutropenia after induction and salvage th, without prophylaxis, and in pts with IC and other fungal infections comparing to IPA.

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COMPARISON OF THE SIDE EFFECTS OF ANTIBIOTICS IN PATIENTS WITH THE DIAGNOSIS OF FEBRILE NEUTROPENIA

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Objectives. Febrile neutropenia is a life-threatening complication that is common in patients with hematological malignancies. It is defined as a single oral body temperature over 38. 3°C or temperature over 38. 0°C lasting at least one hour, in cancer patients with an absolute neutrophil count below 500/mm^3 or in those with an absolute neutrophil count between 500 and 1000/mm^3 with a predicted further decrease within 24-48 hours in the absence of an external cause such as blood products or cytotoxic therapy. High-risk patients should be treated with parenteral antibiotics. **Methods.** Patients with febrile neutropenia who were hospitalized and treated in the hematology clinic of our hospital between January 2005 and December 2011 were retrospectively evaluated. **Results.** Forty (56. 3%) males and 31 (43. 7%) females between 20 and 76 years of age were included in the study. Of the patients, 62. 0% had a diagnosis of acute leukemia and 15. 5% had a diagnosis of lymphoma. Other diagnoses included myeloma, agranulocytosis, hairy cell leukemia and chronic leukemia. One hundred and twenty seven attacks were reviewed. Of these attacks, 74% were experienced by acute leukemia patients. No initial infection foci were identified in 50. 4% of the attacks. In patients with acute leukemia, combination therapy was preferred over monotherapy (p=0. 02). There were no significant differences between monotherapy and combination therapy in terms of efficacy and side effects. Side effects were more common with combination therapy, but the difference did not reach statistical significance. The most commonly preferred drug combination was imipenem plus amikacin (39. 4%). When imipenem plus ciprofloxacin was compared with imipenem plus amikacin, no significant differences were found in terms of efficacy and side effects (p=0. 18). When piperacillin/tazobactam plus ciprofloxacin and piperacillin/tazobactam plus amikacin were compared, side effects were more common in the amikacin group (p=0. 01). When combinations with imipenem and piperacillin/tazobactam were compared, the rate of antibiotic change was 60% and 27. 9% in the piperacillin/tazobactam and imipenem groups, respectively (p=0. 006). Imipenem combinations were more effective in reducing fever within the first 72 hours (p=0. 05). The rate of side effects was 11% with imipenem, 6. 5% with piperacillin/tazobactam, 11. 4% with ciprofloxacin and 16. 9% with amikacin. Side effects were significantly more common among patients using amikacin combinations (p=0. 02). The need for treatment modification was significantly higher in patients with neutropenia lasting for ≥ 10 days (p=0. 003). Side effects were observed during 25 attacks; 11 required antibiotic change. No side effect-related deaths were observed. Ten cases had skin rashes and urticaria, 2 cases had dyspnea and urticaria, 6 cases had nephrotoxicity (hypokalemia in one case), 4 cases had hepatotoxicity, 2 cases had generalized tonic-clonic convulsions and one case had autotoxicity. **Conclusions.** Each hospital should determine an antibiotic regimen appropriate for the bacterial flora of the hospital. In our hospital, imipenem combinations were more effective as empirical combination therapy. In consistent with the literature, there were no significant differences between combination therapy and monotherapy in terms of efficacy. Side effects of antibiotherapy were more common in patients receiving amikacin combinations.

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ORAL SEAWEED CALCIUM DERIVED FROM LITHOTHAMNION IS SAFE AND EFFECTIVE AND WELL TOLERATED AS ORAL LACTATE-GLUCONATE AND CARBONATE CALCIUM IN TUMOR LYSIS SYNDROME WITH RENAL FAILURE AND WATER OVERLOAD.

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Background. Tumor lysis syndrome is a frequent complication in chemotherapy, characterized by hyperkaliemia, hypercalcemia, renal failure, hyperuricemia. Frequently patients need calcium support orally and intravenous. **Aims.** Aim of this study is to verify if in patients with iron overload and with limited intravenous calcium support oral seaweed calcium derived from lithothamnion is safe and effective as oral lactate- gluconate and carbonate calcium. **Methods.** Four patients, two with acute myeloid leukemia, one Burkitt lymphoma and one acute lymphoblastic leukemia presented a tumor lysis syndrome after chemotherapy. All patients were male. The two patients with acute myeloid leukemia were 45 and 52 y. o., the patient with acute lymphoblastic leukemia was 21 y. o., the patient with Burkitt lymphoma was 32 y. o. All patients showed renal failure (CrCl <30 cc/h) and severe hypocalcemia (Ca++ <3. 5 mEq/l). All patients received intravenous support with calcium chloride. The two patients with acute myeloid leukemia received oral lactate- gluconate and carbonate calcium 1 g tid, but the two patients with Burkitt lymphoma and acute lymphoblastic leukemia received oral seaweed calcium derived from lithothamnion 5g tid. **Results.** All patients restored a normal seric calcium level within 9 days. Patients receiving oral lactate- gluconate and carbonate calcium showed abdominal and gastric pain (VAS 7/10) and nausea and vomiting G3 and G4. Patients receiving oral seaweed calcium derived from lithothamnion showed no abdominal and gastric pain and nausea and vomiting G1. **Summary and Conclusions.** Oral seaweed calcium derived from lithothamnion seems to be safe and effective and well tolerated as oral lactate- gluconate and carbonate calcium in tumor lysis syndrome with renal failure and water overload. These data need a confirmation on a larger cohort of patients.

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POST CHEMOTHERAPY FEBRILE NEUTROPENIA IN HEMATOLOGIC MALIGNANCIES: CLINICAL CHARACTERISTICS AND OUTCOME

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Infections in neutropenic patients are a significant reason of morbidity and mortality. We present the clinical features and outcome of 52 febrile neutropenia cases in 42 inpatients of our department during a six month period. Only patients post chemotherapy were included, 18-77 years old. The diagnosis was acute myeloid leukemia in 30 (57%) of them, acute lymphoblastic leukemia in 13 (25%), mixed phenotype acute leukemia in 4 (0. 007%), non Hodgkin lymphoma in 3 (0. 06%), chronic lymphocytic leukemia in 1 and multiple myeloma in 1 of them. Chemotherapy regimens administered were idarubicin - cytarabine in 13 (25%), mitoxantrone - cytarabine in 20 (38%) and etoposide - cytarabine, R-HyperCVAD, R-CHOP and VAD in the rest. Only 1 patient had central venous catheter. During the last three months 19 (36%) had been previously hospitalised. Two (0. 04%) patients were in a single-bed room, 45 (86%) in two-bed room and 3 (0. 05%) in a four-bed. Median neutropenia duration was 14 (8-49) days. The type of infection was identified in 39 (75%) patients, being respiratory in 17 (32%), urinary in 10 (19%), soft tissue in 8 (15%) and bloodstream in 18 (35%). All patients received G-CSF for 11 (5-43) days and had fever for 3 (1-12) days. Gram negative bacteria prevailed in blood cultures. *Burkholderia cepacia*, which caused an epidemic in our department that period was isolated in 8 patients, *Klebsiella pneumoniae* in 3 patients, *Enterobacter cloacae* in 2, *Pseudomonas aeruginosa* in 2, *Acinetobacter baumanii* in 1, *Escherichia coli* in 1 and *Sphingomonas paucimobilis* in one. Only 2 patients had Gram positive blood infection with *Staphylococcus aureus*. Urine cultures showed *Enterococcus spp*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Staphylococcus spp*, *Escherichia coli* and *Candida spp* in one patient each. Mucosal swab cultures showed *Stenotrophomonas maltophila* and *Achromobacter xylosoxidans* in two patients, whereas a patient with neutropenic colitis shed *Acinetobacter baumanii* in stool. *Acinetobacter baumanii*, *Burkholderia cepacia* and two of the *Klebsiella* species were multidrug resistant

strains producing carbapenemases. Death because of infection occurred in 11(21%) patients, in 8 of them attributed to resistant disease and isolation of multidrug resistant strains. Neutropenic fever outcome seems to be affected by primary disease and type of chemotherapy. Patients with acute myeloid leukemia who received anthracycline - cytarabine based regimens had the highest mortality rates in statistical analysis (p = 0. 031 and p = 0. 038 respectively). Moreover mortality tended to be higher in patients during first induction chemotherapy than during subsequent therapies (p = 0. 086).

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PRIMARY PROPHYLAXIS WITH VORICONAZOLE IN ADULTS PATIENTS WITH ACUTE MYELOID LEUKEMIA: 37 CASES

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Background. Invasive fungal infections (IFI) is an important cause of morbidity and mortality in patients with acute myeloblastic leukemia (AML). Patients with hematological malignancies and prolonged neutropenia are at high risk of IFI. The incidence of proven or probable fungal infections ranges from 5-24% among this selected high-risk patients. Deep and prolonged neutropenia in conjunction with some intensive chemotherapies are the main factors that contribute to IFI in this pathology. Because diagnosis is difficult and often delayed, prophylaxis is commonly used as treatment strategy. **Aims.** To evaluated the safety and efficacy of prophylaxis with voriconazole (VOR) in patients with AML. **Methods.** Patients diagnosed of LMA between January 2008 and September 2011 receiving chemotherapy (induction or consolidation) at diagnosis or of relapse, received oral prophylaxis with VOR. This drug was started at the same time to chemotherapy and continued until neutropenia recovery. Dosage was 200mg/12 hours after two loading doses of 400 mg. Patients unable to tolerate oral intake received VOR intravenously. Galactomannan antigenemia was measured twice a week. Neutropenic fever was managed according to IDSA guidelines and IFI diagnosis was defined according to MSG/EORTC criteria. **Results.** Retrospectively we evaluated 28 patients and 37 episodes. Mean age was 53,6 years (19-75), male/female 19/9. The mean days with neutropenia < 0. $1x10^9$/L was 19,4 (0-60) and < 0. $5x10^9$/L was 25,9 (8-60). The type of chemotherapy treatment was ICE (n=13), FLAG-IDA (n=11), LPA-2005 (n=4), others (n=9). Seventeen episodes occurs on induccion fase, 8 on consolidation, and 12 on relapse. Neutropenic fever ocurred in 34/37 (91,8%). Galactomannan antigenemia was possitive in 3 cases (8,1%). Total days with VOR prophylaxis were 29,4 (6-84). The antifungal therapy was modified in 10 cases (27%), most of them for persistent fever. Proven/probable IFI ocurred in 2 cases (5,4%), both of them caused by Aspergillus fumigatus. Toxicity was observed in 3/37 (8,1%), hepatic (1), psychiatric (1) and ophthalmic (1). **Conclusions.** In our experience voriconazole prophylaxis was safe and effective to prevent IFI in this patients, with well tolerance and minimal toxicity.

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HYPEREOSINOPHILIC SYNDROME WITH PULMONARY NOCARDIOSIS

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Abstract. The hypereosinophilic syndromes (HES) are a group of disorders marked by the sustained overproduction of eosinophils, in which eosinophilic infiltration and mediator release cause damage to multiple organs [1-2]. Nocardiosis is an uncommon gram-positive bacterial infection caused by aerobic actinomycetes. Nocardiosis is typically regarded as an opportunistic infection especially in the patients who require prolonged glucocorticoid therapy [3]. We report a rare case of HES with pulmonary nocardiosis and empyema due to using long term corticosteroid. **Case Report.** A 32 year-old male patient was admitted to a local university hospital for dispne, chest pain and hemoptysis 8 months ago. Laboratory examination showed white blood cell count (WBC) 5,71 x 10^9/L, eosinophil 63,9 %. The peripheral blood smear and bone marrow biopsy showed eosinophilia. FIP1L1/PDGFRA rearrangement was positive. He was diagnosed HES and the treatment of high dose prednisolone was started. In the following 5 months prednisolone dose was reduced. Following the reoccurence of the same symptoms, dose was increased again. He was admitted to our hospital in February 2012 with the same symptoms. Physical examination revealed both of the lower zones of the lung sounds reduced and the liver and the spleen were palpable. Laboratory examination showed WBC count 7,8 x 10^9/L, eosinophil 0,2%, erithrocyte sedimentation rate 101 mm/h. In five days he had become febrile. For the treatment of HES imatinib 400 mg/day was started. Pulmonary computed tomography angiography revealed no pulmonary tromboemboly. There was a large pleural effusion (3,5 cm of wideness) in the left hemithorax. There were atelectasis by the pleural effusion. There were necrotic consolidation areas at the left lung upper lobe 8x9 cm and 4x5 cm at the right lung upper lobe. The thoracentesis was performed to the left pleual effusion. The empyema was diagnosed and thorax tube was placed for the drainage. Nocardia growed in the culture of pleural fluid. For the severity of the disease trimethoprim-sulfamethoxazole 3x2 gr/day, imipenem 4x500 mg/day, linezolid 2x600 mg/day were started. During the clinical follow-up his fever was controlled and the complaints disappeared. **Discussion.** Pulmonary involvement and hepatosplenomegaly are usual signs of HES. The best characterized and most frequently observed chromosomal aberration is an interstitial deletion on chromosome 4q12 resulting in the fusion of two genes, that for Fip1-like1 (FIP1L1) and PDGFRA [4]. For all patients with the FIP1L1/PDGFRA mutation (even if asymptomatic), imatinib mesylate is recommended for initial therapy, in preference to other available agents (Grade 1B). Nocardia spp have the ability to cause localized or systemic suppurative disease in immunocomprimised humans and animals [5-6]. Despite the success of TMP-SMX in the treatment of nocardiosis, combination therapy with other agents is warranted in patients with severe infections. In vitro susceptibilities and animal models of disease have demonstrated activity against Nocardia with a variety of antibiotics, including amikacin, imipenem, meropenem, and linezolid [7]

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IS THE USE OF ANTIFUNGAL PROPHYLAXIS THERAPY IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LEUKEMIA NECESSARY?

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Background. Invasive fungal infections (IFI) are serious cases of mortality in neutropenic patients. It is difficult to diagnose IFIs in neutropenic patients and this causes delay in diagnosis which has detrimental effects on prognosis. In order to treat these infections timely, prophylactic, empirical and preemptive treatment strategies are preferred instead of targeted antifungal therapy. **Aims.** The aim of the present study is to evaluate efficiency of empirical and pre-emptive treatment in patients with newly diagnosed acute leukemia without using prophylactic antifungal treatment. **Methods.** Between January 2007 and January 2012, febrile neutropenic attacks of 42 newly diagnosed acute leukemia patients were retrospectively analyzed in Manisa Celal Bayar University, Department of Hematology. In order to commence treatment in an early stage, instead of targeted antifungal therapy, prophylactic, empirical and pre-emptive treatment strategies were implemented. Pre-emptive treatment was considered to be a treatment, given in consideration of a clinical or laboratory diagnosis, which indicates a much higher invasive fungal disease risk in neutropenic patients than other patients. Empirical treatment was considered to be an antifungal therapy, given when patients who had gone under antibiotic therapy more than 5 days and who still had high fever. **Results.** 42 patients with acute leukemia treated with chemotherapy with persistent fever and suspected invasive fungal disease are evaluated in this study. 12 patients received empirical antifungal therapy, 10 patients received pre-emptive antifungal therapy and 20 patients did not receive any antifungal therapy. None of our patients died from invasive fungal diseases. Six patients died from other reasons. Two patients died from acute promyelositic leukemia and disseminated intravascular coagulation and two of them died from leukostasis. One of the remaining two patients died from subarachnoid hemorrhage and the other from cardiac arrest due to congestive heart failure. In empirical antifungal treatment, 7 patients received caspofungin, 3 patients received liposomal amphoterisin, 1 patient received vorikonazol and 1 patient received flukonazol. In pre-emptive treatment, 2 patients received caspofungin, 6 patients received liposomal amphoterisin B, and 2 patients received vorikonazol. In 5 of our patients Candida was found (3 patients Candida Albicans, 1 patient Candida Glabrata and 1 patient Candida Tropicalis). In two patients Aspergillus was isolated and in 3 patients yeast species were found. The average duration of antifungal treatment was 19 days in these series. **Conclusions.** No prophylactic antifungal agents were given to 42 newly diagnosed acute leukemia patients. 22 of those patients were given empirical and pre-emptive antifungal agents. For 20 of our patients antifungal treatment was not needed. None of those 42 patients died due to fungal infection. Studies with larger number of patients are yet needed to take a definitive position about prophylactic antifungal use in the treatment of newly diagnosed acute leukemia.

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IMMUNOHISTOCHEMICAL EXPRESSION OF DNA DAMAGE RESPONSE MOLECULES IN MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA

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Background. DNA double-strand breaks (DSB) may initiate genomic instability, thus contributing to carcinogenesis. Phosphorylation of NBS1, part of the MRE11-RAD50-NBS1 complex, leads to the phosphorylation of ATM; this, in turn, phosphorylates many substrate proteins, including the histone protein H2AX, whose rapid phosphorylation leads to γH2AX foci formation at nascent DSB sites. **Aims.** To assess the immunohistochemical expression of pNBS1, pATM and γH2AX in a series of bone marrow biopsies diagnosed with either myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) and to correlate this expression with the type of MDS, IPSS score and karyotype category. **Methods.** Bone marrow biopsies from 88 patients (73 with MDS and 15 with de novo AML) were used; complete blood count, peripheral blood smear and karyotype analysis were available in all cases. According to the updated (2008) WHO classification, 8 patients were diagnosed with RA, 3 with RARS, 23 with RCMD, 23 with RAEB-1 and 16 with RAEB-2. Sections from paraffin-embedded tissue were immunostained for pNBS1, pATM and γH2AX; at least 1000 marrow cells per case were assessed and the percentage of positive cells calculated. The control group consisted of 20 cases with reactive bone marrow histology. **Results.** The percentage of immunopositive cells for all three molecules was significantly higher in MDS and AML cases, than in reactive marrows (p<0. 05). Statistical analysis showed an overall increasing trend for all immunostain percentages, as disease severity increases. Concerning MDS cases, a higher percentage in high risk compared to low risk MDS cases was seen (p=0. 057 for pNBS1, p=0. 052 for γH2AX and p=0. 025 for pATM). Immunohistochemical expression of pNBS1 was significantly higher in MDS with abnormal versus normal karyotype (p=0. 002). Statistical difference was also found between low versus intermediate karyotype categories (p=0. 01). Moreover, our data suggest that the phosphorylation events of the three different DNA damage response (DDR) proteins (pNBS1, pATM, γH2AX) correlated among each other. In particular, there seems to be a strong correlation between pATM and γH2AX. (p=0. 007, p-values Spearman). **Conclusions.** The aforementioned results suggest that NBS1, ATM and H2AX, being major components of DDR, are activated in MDS and might be involved in AML transformation. Informed consent was obtained by all patients participating in the study. email address of presenting author: maria_kefala@yahoo. com

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MUSASHI2 AND NUMB EXPRESSION IN NORMAL, MDS AND LMA BONE MARROW CELLS

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Introduction. The Musashi2/NUMB pathway has recently been proposed as a potential molecular mechanism to explain differentiation and leukemic progression in myeloid leukemia. By exploiting mouse models of CML, it has been shown that NUMB inhibition, resulting from high levels of MSI2, is essential for development and maintenance of CML-blast crisis. Hence, the overexpression of *MSI2* resulted in increased cell cycle progression and cooperated with the BCR-ABL1 oncoprotein to induce an aggressive immature leukemia. Interestingly, as chronic phase CML progresses to blast crisis, expression of MSI2 is up-regulated and NUMB down-regulated, and modulation of this pathway can inhibit disease, suggesting that MSI2/NUMB pathway participates in disease progression. In AML and CML-blast crisis patients, MSI2 levels were inversely correlated with NUMB expression levels, and higher MSI2 expression was associated with the risk of relapse and death. **Aims.** We hypothesized that MSI2/NUMB pathway may participate in the physiopathology of MDS and its progression to AML. Thus, we characterized the expression of *MSI2* and *NUMB* in healthy control, MDS and in AML and correlated with clinical parameters. **Methods.** Bone marrows from 54 patients with MDS, 38 patients with AML, and from 19 healthy donors were analyzed (Table 1). All healthy controls and patients provided informed written consent and the study was approved by the ethics committee. All patients were untreated at the time of sample collection. *NUMB* and *MSI2* expression levels from total bone marrow were determined by quantitative PCR (q-PCR). **Results.** The comparison between healthy control and the MDS and AML groups for both genes, showed a significant decrease in *NUMB* expression in MDS (*P*=0. 004) and AML (*P*=0. 0001); a significant decrease in *MSI2* expression in the MDS group (*P*=0. 001), and a trend towards a decreased *MSI2* expression in the AML group (*P*=0. 1). *NUMB* expression was significantly lower than that of *MSI2*, in both MDS and AML patient samples (*P*<0. 05), but in the healthy control group, levels were similar. Interestingly, NUMB expression was statistically down-regulated in AML when compared with MDS (*P*=0. 006); the same was not observed for MSI2. *NUMB* and *MSI2* expression did not correlate significantly with clinical parameters (hemoglobin, percentage of blast in BM, number of cytopenias, FAB, WHO classifications and IPPS, for MDS; karyotype for AML), probably because of the small cohort of our study. **Conclusions.** Activation and inactivation of numerous oncogenes and tumor suppressor genes are described in MDS pathogenesis. NUMB has been implicated in cancer as a tumor suppressor; therefore, its down-regulation in MDS and AML, when compared to normal cells and in AML compared to MDS, suggests a role in the pathophysiology of MDS and also the progression of MDS to AML. Further studies are necessary to determine whether this is an independent event in the physiopathology of MDS and AML or if this has an impact on overall survival.

Table 1. Patient and control characteristics.

	Number
Controls	19
Gender	
Male/Female	14/05
Age (yr), median (range)	33 (16-49)
MDS	56
Gender	
Male/Female	35/21
Age (yr), median (range)	66 (90-16)
FAB	
RA/RARS	24/08
RAEB/RAEBt	20/03
LMMC	01
WHO	
RCUD/RCMD/RARS	04/20/08
RAEB-1/RAEB-2	10/10
Myelodisplasia/myeloproliferative	01
AML with myelodisplasia related changes*	03
IPSS	
Low risk/Intermediate-1	15/27
Intermediate-2/High risk	08/04
Not available	02
Cytopenia	
0/1	10/17
2/3	17/12
Dysplasia	
0/1	02/08
2/3	22/24
BM aspirate blast percentage	
<5%	33
5-10%	14
11-19%	06
up 20%	03
Cytogenetics	
Low risk	46
Intermediate/High risk	05/03
Not available	02
AML	35
Gender	
Male/Female	18/17
Age (yr), median (range)	53 (24-93)

BM: Bone marrow; FAB: French-American-British; RA, Refractory Anemia; RARS, Refractory Anemia with Ringed Sideroblasts; RAEB, Refractory Anemia with Excess of Blasts; RAEBt, Refractory Anemia with Excess of Blasts in transformation; WHO, World Health Organization; RCUD, Refractory Cytopenia with Unilineage Dysplasia; RCMD, Refractory Cytopenia with Multilineage Dysplasia; RAEB-1, Refractory Anemia with Excess Blast-1; RAEB-2, Refractory Anemia with Excess Blast-2; AML, Acute myeloid leukemia. IPSS, International Prognostic Score System; INT-1: Intermediate-1; INT-2: Intermediate-2;
* Excluded from the WHO classification analysis.

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WITHDRAWN

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LONG-TERM EVOLUTION OF TYPE III ERYTHROCYTES DURING ECULIZUMAB TREATMENT IN PATIENTS WITH PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

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Background. Patients with paroxysmal nocturnal haemoglobinuria (PNH) are treated with eculizumab, a humanized monoclonal antibody against the com-

plement fraction 5 whose function is to inhibit the membrane attack complex. Usually, after 6 months of eculizumab treatment (MoTT), the number of red blood cells (RBC) bearing a complete deficiency of the glycosylphosphatidylinositol-linked proteins (type III cells) increases significantly. Nevertheless, study of type III RBC remains delicate as some patients still need RBC transfusion during treatment. **Aims.** To appreciate the evolution of type III RBC in patients with PNH clone after at least 12 MoTT. **Methods.** Were included in the study patients without RBC transfusion 2. 5 months at least before testing to rule out the impact of RBC transfusion on the results. A marker, the membrane inhibitor of reactive lysis, was studied on RBC by flow cytometry using a monoclonal antibody, anti-CD59. The presence of CD59- RBC and the size of the clone were evaluated. **Results.** Eight patients were selected: 4 with aplastic anaemia (AA) and PNH clone (n° 1-4), one of them (patient n°3) was transfusion dependent, and 4 patients with PNH (n°5-8), patient n° 5 suffering also from thrombocytopenia. Before treatment, CD59- RBC were at 12. 9%, 6. 1%, 51. 0% and 6. 6% in AA patients n°1 to 4, respectively. During treatment, the percentage of CD59- RBC decreased to 2. 7% in patient n°2 (31 MoTT) and to 1. 7% in patient n°4 (18 MoTT) and increased in the two other patients [n°1: 42. 7% (28 MoTT) and n°3: 95,8% (31 MoTT)]. In all patients with PNH, the size of the CD59- RBC clone had increased: from 4. 0% before treatment to 7. 1% in patient n°5 (42 MoTT), from 9. 5% to 13. 8% in patient n°6 (25 MoTT), from 17. 8% to 51. 1% in patient n°7 (27 MoTT) and from 1. 0% to 5. 5% in patient n°8 (27 MoTT). An increase was observed in patient n°5 despite a clinical course of AA. **Summary and Conclusions.** Two criteria were selected for this study: a long period of eculizumab treatment (over 12 months) and the absence of RBC transfusion 2. 5 months at least before testing. Results are heterogeneous in AA patients with PNH clone. A decrease of the CD59- RBC clone size was observed in half of these patients, which leads us to express that for this kind of patients this parameter does not seem a good marker to assess the efficiency of the treatment. Conversely, in PNH patients the size of the CD59- RBC clone increased: here this parameter could be used for the treatment monitoring provided that the two conditions of the study are respected.

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HEDGEHOG PATHWAY IS DEREGULATED IN MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA

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Background. Studies regarding self-renew in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) show that blast colony formation is not confined to AML, but also occurs in patients with advanced MDS. Hedgehog pathway have an important role in self-renew of normal and leukemic stem cells and is upregulated in myeloid leukemias, however there are no studies regarding Hedgehog pathway in MDS. Hedgehog ligands (Sonic hedgehog [SHh], Indian hedgehog [IHh], and Desert hedgehog [DHh]) are produced by stromal cells and bind to the receptor Patched (PTCH). This binding causes activation of Smothened (SMO) receptor, resulting in downstream transcription of target genes. **Aims.** To evaluate Hedgehod pathway components in MDS and AML. **Methods.** Bone marrow (BM) samples were collected from 39 MDS (25 low risk,14 high risk-WHO 2008) patients and 26 healthy donors. Relative expression of PTCH and SMO were obtained by Real Time PCR. For immunohistochemistry, BM biopsies were collected from 4 AML patients, 3 MDS and 6 megaloblastic anemia (MA), used as control. The BM sections were stained with antibodies for IHh and DHh (1:50), the percentage of stained nucleated cells based on an average of 5 high-powered fields were considered. The acute myeloid cell lineage U937 was cultured with 15μM of cyclopamine, a Hedgehog inhibitor, or tomatidine, as control, for 24 and 48 hours followed by analysis of apoptosis by Annexin-PI staining, cell cycle by PI staining and proliferation by MTT assays. For clonogenic assays, U937 was maintained in methylcelulose for 20 days in the presence of 15 μM of cyclopamine or tomatidine. **Results.** Hedeghog receptors PTCH and SMO were overexpressed in MDS BM cells compared to normal BM as follow; PTCH: [median (min-max) healthy donors= 2. 23 (0. 42-9. 22); low risk= 6. 09 (0. 41-25. 28); high risk= 3. 97 (0. 42- 21. 01); healthy donors *vs* low risk p= 0. 02; healthy donors *vs* high risk p= 0. 02]; SMO: [healthy donors= 2. 33 (0. 00-16. 11); low risk= 14. 79 (1. 89- 41. 93); high risk= 34. 44 (8. 04- 164. 28); healthy donors *vs* low risk and healthy donors *vs* high risk p<0. 001]. In immunohistochemistry assays, the expression of Hedgehog ligants was also higher in AML BM cells and MDS when compared to control cells. For IHh, the number of stained cells in MDS were twice higher than MA (p=0. 02) and for DHh, the increase in AML *vs* MA was 4 folds (p= 0. 02). In order to investigate if Hedgehog inhibition affects self-renew in myeloid cells, we treated U937 cells, which express all components of this pathway, with cyclopamine and observed no affect in apoptosis, but cell accumulation in G1 phase (p=0. 01) and decrease of cell number in S phase (p=0. 0002). The cell cycle arrest reflected in 34% of proliferation decline (p=0. 007). In methylcellulose assays, cyclopamine causes abolishment of colony formation. **Conclusions.** As far as we know, this is the first study of the Hedgehog pathway in MDS. We observed overexpression of Hedgehog ligands and receptors as well as abolishment of colony formation after the pathway inhibition. Deregulation of Hedgehog in MDS and AML may be related to abnormal self renew.

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EFFECT OF LENALIDOMIDE TREATMENT ON THE EXPRESSION OF FLI1, EKLF, TP53, HDM2, PU. 1 AND IL-6 GENES IN 5Q- SYNDROME

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Background. Lenalidomide has improved the treatment of MDS with 5q deletion, inducing a hematologic (erythroid) and cytogenetic response in the majority of patients. The exact mechanisms of lenalidomide action explaining its effects on erythropoiesis are not clear thus far. We recently studied two transcription factors Fli1(Friend leukemia virus integration 1) and EKLF (Erythroid Krüppel like factor) involved in MEP (common megakaryocytic and erythroid progenitor) differentiation in MDS patients and showed significant decrease of EKLF and increase of Fli1 in 5q- syndrome (Neuwirtova et al, ASH 2011). There exists cross-antagonism between Fli1 and EKLF. Fli1 mRNA is target for microRNA-l45 (miR-145) localized in common deleted region of 5q. Haplo-insufficiency of miR-145 in 5q- syndrome stabilizes Fli1 mRNA and increases Fli1. Fli1 is also increased by IL-6. IL-6 is induced by haplo-insufficiency of miR-145 and miR-146a. Transcription factor PU. 1 is positive regulator of *Fli1* gene expression. In mice Fli1 regulates p53 via MDM2 (mouse double minute 2 or HDM2 in humans), an E3 ubiquitin ligase, which promotes p53 degradation in proteasomes. **Aims.** To study the changes in expression of above mentioned genes, which are conjectured to play a role in the pathogenesis of 5q- MDS, during the lenalidomide therapy course. **Methods.** We studied the expression of *Fli1*, *EKLF*, *TP53*, *HDM2*, *PU. 1* and *IL6* genes, which are supposed to play a role in the pathogenesis of 5q- syndrome, in the course of lenalidomide therapy in mononuclear cells isolated from peripheral blood of twelve MDS patients with 5q- (9 females, 3 males) of a median age of 70 years (range 55-78 years). Informed consent was obtained from all patients. All were transfusion dependent before treatment. Levels of specific mRNAs were determined by quantitative real-time PCR in total RNA isolated from blood. Relative levels of specific mRNAs were calculated to the level of housekeeping GAPDH mRNA. **Results.** The beneficial effect of lenalidomide was seen in 10 patients. Lenalidomide promoted induction of hemoglobin synthesis, which reflected the increase of EKLF mRNA levels and on the contrary decrease of the Fli1 mRNA. The decline in Fli1 mRNA levels was accompanied with the decrease of the platelet counts. The levels of Fli1 mRNA and EKLF mRNA oscillate after the normalization of hematopoiesis in the course of further lenalidomide therapy. Levels of p53 mRNA surprisingly did not show any clear decrease after lenalidomide treatment. On the contrary, levels of PU. 1 mRNA and IL-6 mRNA consistently increased in the majority of patients. HDM2 mRNA levels were lowered after lenalidomide treatment in most of examined 5q- patients. **Conclusions.** We can conclude that lenalidomide treatment is associated with elevation of EKLF mRNA levels and with decrease of Fli1 mRNA levels in majority of treatment-sensitive MDS patients with 5q- syndrome, which reflects the improvement of erythropoiesis, increase of hemoglobin and decrease of platelet production. Unexpectedly, we found consistent decrease of HDM2 expression, but any changes in TP53 expression and surprisingly PU. 1 and IL6 expression increased in almost all our patients, contrary to our expectations subsequent to others authors reports.

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USEFULNESS OF TRANSFERRIN SOLUBLE RECEPTOR DOSAGE AS A MARKER OF DYSERYTHROPOIESIS IN LOW RISK MDS

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Background. Transferrin soluble receptor (Tf-SR) dosage is a useful tool to assess iron deficiency but it could also reflect the level of erythropoietic activity correlated with erythroblastic pool. **Aims.** We hypothetized that Tf-SR could be a marker of dyserythropoiesis in myelodysplastic syndromes (MDS). We decided to investigate the potential links between Tf-SR with (i) bone marrow erythroblastic pool, and (ii) other parameters such as: polychromatophilic erythroblast

(PCE) pool, MDS subtype, IPSS, endogenous EPO level, ferritin, reticulocytes response to EPO treatment if available. **Methods.** we reviewed 125 MDS patients, 65 were excluded (18 due to previous blood transfusion, 6 due to iron deficiency, 4 due to vitamin deficiency, 15 due to additional bone marrow failure, 7 due to uncertain diagnosis of MDS, 5 due to unavailable slides (bone marrow aspirate), renal failure (clearance of creatinin < 30ml/min). The 60 remaining patients were evaluated for dyserythropolesis by two different cytologists according to a pre-definite tool. Moreover two other groups were evaluated including 11 healthy subjects and 13 anaemic subjects of various causes (iron deficiency, B9 deficiency, renal failure). **Results.** 37 men and 23 women were included; mean age was 73. 3 years (56-93), including 5 RC, 13 RARS, 3 5q minus syndromes, 11 RCMD, 11 RCMD-RS, 10 RAEB-1et 7 RAEB-2. For 57 patients cytogenetic analysis was available, normal in 41 patients. A positive correlation between dyserythropoiesis and Tf-SR level was found in low risk MDS but not in high risk MDS. **Conclusions.** our study showed a positive correlation between Tf-SR level and erythroblastic activity as well as dyserythropoiesis intensity. Subsequent studies are needed to evaluate the Tf-SR during the course of the disease particularly on EPO therapy and to assess the prognosis value of this marker.

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METHYLATION CHANGES IN CDKN2B AND CDKN2A GENES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA

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Background. Epigenetic *de novo* methylation of CpG islands is thought to be one of the key moments in malignant transformation of the cell. The most frequently tested genes are *CDKN2B* gene (cyclin-dependent kinase inhibitor gene) and its functional homologue *CDKN2A* gene. *CDKN2A* gene generates several transcript variants which differ in their first exons (isoform 1 and isoform 4). **Aims.** The methylation status of the *CDKN2B* and *CDKN2A* genes was studied in DNA samples isolated from bone marrow mononuclear cells (BM)-MNCs in Czech patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) before treatment and during the course of the disease to evaluate its prognostic significance and association with clinical outcomes and disease progression. **Methods.** In our study methylation of these genes was studied in 63 patients with MDS, 2 with myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and 13 with AML. Five patients were monitored during 5-azacytidine treatment. Twenty-six DNA samples from healthy donors (HD) were used as non-malignant controls. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) method with all associated techniques was used for detection. MS-MLPA kit enables simultaneously detect copy number variations and methylation changes of genes located in *CDKN2A-CDKN2B* region at 9p21. **Results.** We found that abberant methylation was present in the *CDKN2A* gene in 38% and in the *CDKN2B* gene in 77% of the patients in MDS group. The level of methylation was higher in the group of AML patients - 77% in *CDKN2A* gene and 100% in *CDKN2B* gene. In MDS patients, an aberrant methylation was associated with a tendency to disease progression towards more advanced forms according to the World Health Organization (WHO) classification and the International Prognostic Scoring System (IPSS). Significant differences in methylation level were observed between early and advanced forms of MDS in *CDKN2B* gene (P value < 0. 05) but not for *CDKN2A* gene. The trend of methylation in patients treated with azacitidine was analyzed in *CDKN2B* gene and correlated with the course of the disease. Increased methylation was connected with disease progression. **Summary.** We concluded that the methylation level of *CDKN2B* gene might be used as a marker of leukemic transformation in MDS. Our study indicates the role of hypermethylation as an important event in the progression of MDS to AML. Supported by the grant of MHCR 00023736.

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DIFFERENTIAL EXPRESSION OF AURKA AND AURKB GENES IN BONE MARROW STROMAL MESENCHYMAL CELLS (MSCS) OF MYELODISPLASTIC SYNDROME (MDS): CORRELATION WITH G-BANDING ANALYSIS, SKY AND FISH

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Background. It has been demonstrated that genomic abnormalities of cells in the hematopoietic microenvironment could induce MDS and subsequent transformation to AML. Whether abnormalities associated with MSCs may contribute to pathogenesis of MDS and leukemias, and subsequently disease progression it is still not totally clear. **Aims**. In the present study we compare the expression profile of *AURKA* and *AURKB* in hematopoietic cells (HC) and MSCs of 48 MDS patients and 16 healthy donors (HD). Secondly, we correlate the cytogenetic findings obtained, with expression profile of *AURKA* and *AURKB.* **Methods**. Mononuclear cells (MNCs) were isolated from BM samples during initial diagnosis. MNCs from MDS and HD were cultured and collected after 3-4 passages for subsequent G-banding analyses, SKY, FISH, and RNA isolation. We also applied a panel of "MDS FISH probes"[t(3;3)/inv(3q), 5q-, 7q, +8 and 20q-] on MSCs in order to search for specific abnormalities of MDS. **Results**. We stratified the patients according the cytogenetic profile (normal vs. abnormal), for each group (HC and MSCs) and significant differences were observed (*AURKA* [mean value of $2^{-\Delta\Delta Ct}$±SD]: 3,649 ± 0,1059 N=18 vs. 4,604 ± 0,2743 N=23, p < 0,0001, in HD vs. normal karyotype HC; *AURKA* [mean value of $2^{-\Delta\Delta Ct}$±SD]: 3,649 ± 0,1059 N=18 vs. 7,992 ± 0,2416 N=32, p < 0,0001, in HD vs. normal karyotype MSCs; *AURKA* [mean value of $2^{-\Delta\Delta Ct}$±SD]: 4,604 ± 0,2743 N=23 vs. 14,70 ± 0,5661 N=25, p < 0,0001, in normal karyotype HC vs. abnormal karyotype HC; *AURKA* [mean value of $2^{-\Delta\Delta Ct}$±SD]: 7,992 ± 0,2416 N=32 vs. 11,72 ± 0,4951 N=16, p < 0,0001, in normal karyotype MSCs vs. abnormal karyotype MSCs). The higher expression of *AURKA* in HC and MCS, with altered karyotype versus normal karyotype, in the same population was also confirmed by FISH using a commercial probe for *AURKA* gene. We also found significant differences in HD vs. normal karyotype HC and normal karyotype MSCs for *AURKB*, respectively (*AURKB* [mean value of $2^{-\Delta\Delta Ct}$±SD]: 1,587 ± 0,05907 N=18 vs. 2,830 ± 0,1137 N=23, p < 0,0001 and vs. 3,082 ± 0,08036 N=32, p < 0,0001). However, when we compared normal vs. altered karyotype for HC and MSCs we found no difference between the groups. In MSCs with chromosomal abnormalities (CA), the karyotypes observed were different from those of their HC. FISH analysis on MSCs by using a "MDS FISH probes"revealed no MDS abnormalities in MSCs. Clonal abnormalities seen in MSCs by G-banding analysis were also confirmed by SKY and this observation confirms that CA showed in MSCs, from MDS patients, are not random. **Summary.** This investigation is the first attempt to correlate the gene expression profile of *AURKA* and *AURKB* in a cytogenetically stratified population of MSCs from MDS patients. In fact, both cells (HC and MSCs) may probably be altered in response to damage-inducing factors, and the presence of genomic abnormalities in MSCs suggests that an unstable bone marrow microenvironment may facilitate the expansion of MDS/leukemic cells. Financial support: FAPESP (Proc. 2007/52462-7 and 2011/01647-2)

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DOWNREGULATION OF SPINT2 IN MYELODYSPLASTIC SYNDROMES MAY BE RELATED TO ABNORMAL MICROENVIRONMENT

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Background. Myelodysplastic syndrome (MDS) encompasses a group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis resulting in impaired differentiation and peripheral blood cytopenias. In MDS, there are evidences of defective bone marrow stromal cells supporting hematopoiesis, affecting development and apoptosis of hematopoietic stem cells. Microarray analysis of bone marrow stromal cells from patients with the MDS subtype refractory anemia with ringed sideroblasts (RARS), conducted by our group, identified low expression of *SPINT2 (*serine peptidase inhibitor) compared to normal bone marrow stromal cells. *SPINT2* corresponds to a matriptase which has tumor suppressor function, acting upon the inhibition of cell growth and induction of apoptosis by balancing between BAK and BCL2 proteins. SPINT2 downregulation was associated with prognostic and progression of solid tumors, including breast, cervical, hepatocellular carcinoma and medulloblastoma, nevertheless, there are as yet no studies on SPINT2 expression in MDS. **Aims.** Characterize SPINT2 expression in bone marrow stromal cells from normal donors and MDS patients. Furthermore, we evaluated the expression levels of SPINT2 in bone marrow cells from normal donors and MDS patients, compared among low-risk and high-risk MDS and correlated with laboratorial and clinical data. **Methods.** A total of forty-two patients with diagnosis of MDS, receiving no treatment, were included in the study; twenty-six samples from normal donors were used as controls. Patients were grouped into low-risk and high-risk disease, according to FAB, WHO classification and IPSS (Table 1). This study was approved by the National Ethical Committee Board. The stromal cells from MDS patients and normal donors were evaluated by FACS for the absence of CD34, CD45 and CD68 antigens after bone marrow mononuclear cells were isolated by Ficoll Hypaque gradients. Total bone marrow cells were submitted to RNA extraction after removal of erythrocytes by hemolysis. SPINT2 expression levels were determined by quantitative PCR. Data were expressed as the median [minimum-maximum]. For comparisons Mann-Whitney test was used. Spearman correlation analysis was used for ranking correlation tests. P-value <0. 05 was consid-

ered as statistically significant. **Results.** In stromal cells, we observed that the *SPINT2* expression was significantly decreased in MDS patients when compared with normal donors (0. 46 [0. 08-0. 77] versus 1. 16 [0. 76-1. 41], respectively; *P*=0. 005). Therefore, we found a significant decrease in *SPINT2* expression of total bone marrow MDS cells compared with normal cells (0. 83 [0. 03-4. 05] versus 1. 85 [0. 22-7. 60], respectively; *P*=0. 017). *SPINT2* expression was lower in both low-risk and high-risk patients when compared to normal subjects and demonstrated a significant positive correlation with peripheral blood neutrophils (*r*=0. 31, *P*=0. 04) and bone marrow granulocytes percentages (*r*=0. 31, *P*=0. 04). No correlation with age, hemoglobin, platelet, cytopenia, and blast percentage in bone marrow was found. **Conclusions.** *SPINT2 is* downregulated in stromal and total bone marrow cells and correlates with granulocyte number in MDS. These findings suggest a possible role of SPINT2 in MDS physiopathology and microenvironment abnormalities. Therefore, SPINT2 studies could help to better understand MDS bone marrow microenvironment and may be useful in the development of therapeutic strategies or as a potential source of biomarkers. Supported by FAPESP, CNPq and INCTS do Sangue.

Table 1. Patient characteristics.

	Number
MDS Patients	42
Gender	
Male/ Female	17/15
Age (years), median (range)	69 (16-86)
FAB	
RA/RARS	16/9
RAEB/RAEBt	15/2
WHO	
RCUD/ RCMD/RARS	2/11/6
RAEB-1/RAEB-2	8/4
AML with myelodisplasia-related changes*	3
IPSS	
Low-risk/ INT-1	12/21
INT-2/ High Risk	5/1
Not avaliable	2
Cytogenetics	
Normal	38
Monosomy 7	1
-Y	1
Complex	1
Not avaliable	1
Cytopenia	
0 and 1 cytopenia	22
2 and 3 cytopenias	20

Abbreviations. MDS: Myelodysplastic Syndrome; FAB: French-American-British; RA, Refractory Anemia; RARS, Refractory Anemia with Ringed Sideroblasts; RAEB, Refractory Anemia with Excess of Blasts; RAEBt, Refractory Anemia with Excess of Blasts in transformation; IPSS, International Prognostic Score System; INT-1: Intermediate-1; INT-2: Intermediate-2; WHO, World Health Organization; RCUD, Refractory Cytopenia with Unilineage Dysplasia; RCMD, Refractory Cytopenia with Multilineage Dysplasia; RAEB-1, Refractory Anemia with Excess Blast-1; RAEB-2, Refractory Anemia with Excess Blast-2; AML, Acute myeloid leukemia. *Excluded from the WHO classification analysis.

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INVESTIGATION OF OCT4A AND TOTAL OCT4 IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES(MDS) AND ACUTE MYELOGENOUS LEUKEMIA (AML)

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Background. The transcription factor OCT4 is an established regulator of stemness and pluripotency in embryonic stem cells. It has been postulated that reactivation of its expression occurs in somatic stem cells that have undergone carcinogenesis. The isolation of cDNAs encoding the OCT4 gene, demonstrated the existence of two isoforms of this transcription factor termed OCT4A and OCT4B that are generated by alternative splicing. The stemness properties are attributed to OCT4A, while OCT4B, localized in the cytoplasm, is currently considered of unknown function. Interestingly a novel OCT4 spliced variant has been recently discovered, OCT4B1, which has been also considered as a putative marker of stemness. The role of OCT4 isoforms in myeloid malignancies has been poorly studied. AIM: To investigate the RNA expression of OCT4A and of total OCT4 containing all variants in MDS and AML patients. **Methods.** Bone marrow aspirates from fifty seven patients, 27 RA/RARS/RCMD (group A), 13 RAEB1/RAEB2 (group B), 17 AML (group C) were studied. Relative expression of the above transcripts was assessed on total RNA with reverse transcription followed by real-time polymerase chain reaction (PCR). Special primers and probes were used in order to distinguish between the two different products. The data obtained were analyzed by using Kruskal-Wallis statistics. **Results.** The median relative expression level of OCT4A in group A was 0. 005(range: 0- 0. 72) and for groups B and C the median values were 0. 0003 (range: 0 - 0. 29) and 0. 008 (range: 0-0. 4) respectively. The median relative expression level of total OCT4 containing all variants in group A was 0. 63 (range: 0 - 48. 4) and for groups B and C the median values were 0. 17 (range: 0. 05-4. 05) and 0. 29 (range: 0-48. 9) respectively. Relative expression levels of both, OCT4A and total OCT4, were not significantly different among patient groups (p >0. 05, Kruskal-Wallis). **Conclusions.** Our results show that OCT4A and alternatively spliced variants are expressed in the haematopoietic compartment. The considerable difference among the values of the two measures, underlines the importance of a distinct and precise reference of the identity of different OCT4 derivatives. Although our results indicate that stemness properties of OCT4A may not contribute to the progression of MDS to AML more research is needed to elucidate the hypothesis that alternatively spliced transcripts of OCT4 may be implicated in MDS and AML pathogenesis.

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SIVA, A PROAPOPTOTIC PROTEIN, IS DOWNREGULATED IN MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA

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Background. The p53 tumor suppressor protein is a key transcription factor that regulates several signaling pathways involved in the cell response to stress, suppressing malignant transformation by cell cycle arrest, DNA repair, induction of apoptosis or initiation of senescence. Downregulation or inactivation of p53 is a common event in hematological malignancies, including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) and has been related with poor prognosis. SIVA was initially described as a proapoptotic protein. In acute leukemia cell lines, both isoforms of SIVA (SIVA1 and SIVA2) play an important role in the apoptotic pathway induced through CD27 antigen by inhibition of BCL-XL, with consequent release of cytochrome C and caspases 9 and 3 activation. Additionally, activation of SIVA is capable of inhibiting NFkB and its role up the activation of BCL2 and BCL-XL. *SIVA* has recently been described as a transcriptional target of p53 and SIVA1 binds to p53 and modulates its stability. Despite the evidence that SIVA is an apoptosis-selective p53 target gene, the study of SIVA in primary hematopoietic cells has not been performed. **Aims.** To characterize *SIVA1*, *SIVA2 and TP53* expression in bone marrow cells from healthy donor, MDS and AML patients. **Methods.** We studied 22 healthy donors, 40 patients with MDS (FAB: 23 low-risk [RA/RARS] and 17 high-risk [RAEB/RAEBt]; WHO: 23 low-risk [RCUD/RCMD/RARS] and 14 high-risk [RAEB-1/RAEB-2]) and 29 AML patients at the time of diagnosis. *SIVA1, SIVA2* and *TP53* transcripts levels were determined by quantitative-PCR. Data were expressed as the median [minimum-maximum]. *Mann-Whitney* test and Spearman correlation analysis were used. **Results.** *SIVA1* and *SIVA2* transcripts were significantly decreased in MDS and AML cells compared with normal cells (*SIVA1*: 0. 79 [0. 15-10. 28], 0. 93 [0. 21-2. 23] *vs.* 2. 18 [0. 23-25. 88], respectively, *P*<0. 0001; *SIVA2*: 0. 99 [0. 29-3. 98], 0. 89 [0. 38-18. 14] *vs.* 4. 69 [0. 81-35. 53], respectively, *P*<0. 0001). No difference was observed between low-risk, high-risk MDS and AML. Spearman correlation analysis showed a significant positive correlation between *SIVA1* and *SIVA2* expression in normal (*r*=0. 74, *P*<0. 0001), MDS (*r*=0. 80, *P*<0. 0001), and AML (*r*=0. 81, *P*<0. 0001) cells, indicating a similar regulation for both isoforms of SIVA in hematopoietic cells. *TP53* expression was similar between normal, MDS and AML cells (1. 10 [0. 04-7. 32], 1. 03 [0. 03-33. 41] and 1. 53 [0. 27-9. 09], respectively); a slight increase in *TP53* was observed in AML comparing to MDS cells (*P*=0. 01). No correlation between *SIVA* and *TP53* expression were

noted. **Conclusions.** *SIVA1* and *SIVA2* expression are downregulated in MDS and AML cells compared to normal cells, suggesting that defective *SIVA* expression may be involved in the physiopathology of these diseases. *TP53* expression did not differ among the normal and malignant hematopoietic cells and did not correlate with *SIVA* expression. Other mechanisms of p53 regulation, as mutation, protein phosphorylation and degradation may contribute to the regulation of *SIVA* in MDS and AML cells. Induction of SIVA in MDS and AML cells may be attractive for these diseases, since SIVA upregulation has been associated with induction of apoptosis of leukemia cell lines.

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CYTOGENETIC CATEGORIES OF MYELODYSPLASTIC SYNDROMES (MDS) ACCORDING TO REVISED INTERNATIONAL PROGNOSTIC SCORING SYSTEM (R-IPSS)

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Background. The biological and clinical heterogeneity of MDS is the reason for further improvement of the stratification of MDS according to the risk of AML transformation and overall survival. **Aims.** To analyze the distribution of 197 de novo MDS patients with the agreement of R-IPSS karyotype. **Methods.** Standard Karyotyping. **Results.** The median age of patients was 64 y. o. (14-86). The bone marrow blasts was less than 5% in 90 patients (45. 7%). There was the correlation between age and BM blasts ≥5% (r=0. 159; p=0. 024). The chromosome aberrations were revealed in 129 patients (65. 5%). Next variants of karyotype were the common: normal (34. 5%), complex with >3 aberrations (19. 3%), not otherwise specified single and double abnormalities (16. 2%), del(5q) (10. 7%) and -7/7q- (6. 1%). Other chromosome abnormities referred to R-IPSS have been found in single patients or were not found at all, e. g. +19 or +21 as single aberrations. The karyotype of 2 patients with double aberrations and 22 patients with multiple lesions was in line with monosomal karyotype which is not mentioned in R-IPSS. The association of normal and complex karyotype with BM blasts (<5% and ≥5%) was determined (r=0. 469; p=0. 000). The distribution of patients according to R-IPSS prognostic variants of karyotype was next: very good (3. 0%), good (48. 2%), intermediate (25. 9%), poor (3. 6%) and very poor (19. 3%). In accordance with IPSS the karyotype of 52/197 patients (26. 4%) was categorized as intermediate one that is without any additional definition. And there were fewer patients with single or double abnormalities that were not otherwise specified and included in intermediate variant of R-IPSS karyotype - 32/197 (16. 2%). The last group was represented with 29 different chromosome aberrations as a sole abnormality in most of them. **Conclusions.** Not numerous contents of some R-IPSS variants and retained multiple chromosome lesions with undetermined prognosis are the reasons to look for new biological prognostic markers, e. g. genetic lesions.

1450

THE EFFECT OF CYCLINA1 ANTISENSE OLIGODEXYNUDEOTIDE ON PROLIFERATION REGULATION OF MYELODYSPLASTIC SYNDROME CELLS

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Background. The physiological role and the expression of cyclinA1 is different in various organisation cells or in tumor cells from different sources. Some reports showed that the overexpression of cyclinA1 could promot the growth of tumor cells, and the antisense structure could inhibit cell growth. **Aims.** To investigate the effect of cyclinA1 antisense oligodeoxynudeotide(ASODN) on mediating proliferation of myelodysplastic syndrome cells(MUTZ-1). **Methods.** CyclinA1 ASODN that complementary to the transcriptional initiation of cyclinA1 mRNA were used in vitro culture MUTZ-1 cell study. The protein and mRNA expression levels of cyclinA1 were detected by immunohistochemical method and RT-PCR et al. The cell apoptosis was detected by several methods. Electron microscopy, In site cell apoptosis detection kit, DNA gel electrophoresis and FCM . **Results.** In ASODN group, the protein or mRNA expression levels of cyclinA1 were significantly inhibited than those in control group (p<0. 01). The ratio of MUTZ-1 cell proliferation and CFU-MUTZ-1 were significantly inhibited. Morever, the inhibitory ratio was significantly increasing as the dose of ASODN increased. The apoptosis ratio□5. 97±0. 81%□in ASODN group was significantly higher than control group (1. 81±0. 72%)(P<0. 01). The morphologic study of MUTZ-1 cells in ASODN group showed smaller nuclei, few cytoplasm, lesser mitotic figure and lasser degree of atypia compared to the control group. The electron micrographs of MUTZ-1 cells in the ASODN group showed the characteristics of apoptosis (the apoptosis body): the chromatin condensed in masses at the periphery of nucleus, leaving the center filled with an electron-opaque material with mitochondria pyknosis in cytoplasm, and the nuclear fragmentation were seen, and the endoplasic reticulum was slightly extended in the condensed cytoplasm, but they were not seen in control group. We could also detect the DNA ladder in ASODN group. **Conclusions.** CyclinA1 ASODN with liposomal transfection has high inhibit the proliferation efficiency to the MUTZ-1 cells in vitro and has effect to induce the myelodysplastic syndrome cells to apoptosis. It can specifically inhibit it's protein and mRNA expression levels as well as the leukemia cell proliferations, which effect depends upon the concentration of ASODN. CyclinA1 have been demonstrated to play an important role in some biological phenomena such as proliferation and apoptosis, so cyclinA1 maybe a potential target for gene therapy in myelodysplastic syndrome.

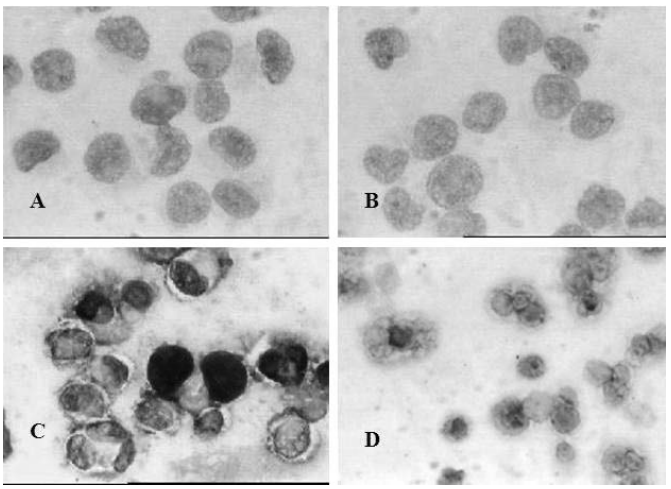

Figure 1. Apoptosis cells were detected by POD(×400). A: Control group; B: SODN group; C&D: ASODN group.

1451

INCREASED FREQUENCY OF THE MUTANT ALLELE G OF TLR4 RS4986790 POLYMORPHISM IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. In patients with myelodysplastic syndromes (MDS), infection vulnerability does not always reflect the severity of neutropenia. This observation suggests that additional factors may predispose MDS patients to infections. **Aims.** Based on available evidence suggesting that the rs4986790 and rs4986791 Single Nucleotide Polymorphisms (SNP) of human toll-like receptor (*TLR)-4* may be associated with increased susceptibility to bacterial infections, we sought to examine the possible association between *TLR4* SNPs and infection susceptibility in MDS patients from a homogeneous Greek population. **Methods.** Genomic DNA was extracted from the bone marrow of 74 MDS patients and 221 unrelated healthy controls from the island of Crete. Genotyping for TLR4 rs4986790 and rs4986791 SNPs was performed by PCR - Restriction Fragment Length Polymorphism (RFLP), using the *Nco*I and *Hinf*I restriction enzymes, which digest the G allele-containing and T allele-containing PCR products for the rs4986790 and rs4986791 SNPs, respectively. Both undigested and digested PCR products were visualized in 3% agarose gel stained with ethidium bromide. To examine the possible correlation of the rs4986790 and rs4986791 polymorphic genotypes with MDS patient susceptibility to infections, we recorded the number of infections per patient tested, since the day of diagnosis. **Results.** No statistically significant difference was found in the frequency of infectious episodes between MDS patients carrying the risk allele and the MDS patients that did not carry the risk allele for either SNPs. This is probably explained by more recent data which show that only the mutant rs4986790/wild type rs4986791 haplotype is considered to correlate with susceptibility to Gram- infections, while the double heterozygous haplotype (mutant rs4986790 and mutant rs4986791) is considered to have a neutral phenotype which is indistinguishable from that of the wild type *TLR4.* Interestingly, among healthy controls, all carriers of the rs4986790 SNP also carried the minor allele of rs4986791, showing double heterozygosity for

rs4986790 and rs4986791. However, 28. 6% of MDS patients with the polymorphic genotype of rs4986790 had the wild-type genotype of rs4986791. Interestingly, the frequency of the minor allele G of the rs4986790 SNP was found significantly increased in MDS patients (4. 7%) compared to healthy controls (1. 6%) (P=0. 0295). Furthermore, the heterozygous polymorphic genotype A/G seemed to be more frequent among MDS patients (9. 5%) compared to controls (3. 2%) (P= 0. 0276). The frequency of the minor allele T and the polymorphic genotype C/T of rs4986791 SNP was also higher in MDS patients, although not at a statistically significant level. **Summary and Conclusions.** The presence of the polymorphic genotype of rs4986790 does not seem to correlate with MDS patients' vulnerability to infections. However, the frequency of the mutant allele G and the A/G genotype is significant higher in MDS patients compared to healthy controls, representing probably a genetically defined risk factor for MDS development.

1452

THE IMPACT OF OLD AND NEW PROGNOSTIC FEATURES ON THE SURVIVAL OF PATIENTS WITH MDS

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Background. The evaluation of prognostic features in patients with myelodysplastic syndromes (MDS) has been important, due to the wide variability in clinical course, as well as for the evaluation of new treatment options. IPSS has been widely used, but the importance of cytogenetic findings was recently revised. Also, the prognostic importance of anemia and transfusion dependency has been recognized and included in the WPSS score. On the other hand, flow cytometry has been recently standardized for diagnosis of MDS, and the prognostic importance of some phenotypic features has been repeatedly shown. **Aims.** In a prospective study, we compared the influence of peripheral blood (PB) counts, bone marrow (BM) features, IPSS, WPSS, revised cytogenetic classification, and phenotypic abnormalities on the overall survival of patients with primary MDS. **Methods.** We studied 87 cases diagnosed by WHO criteria. BM immunophenotyping was performed using a four-color panel of monoclonal antibodies analyzing the mielomonocytic series. **Results.** mean age: 65 years. WHO types: 6 cases were RA, 1 5q- syndrome, 40 cases were RCMD, 13 cases were RCMD-RS and 27 were RAEB. According to IPSS, 32 were low risk, 32 were intermediate-1, 12 were intermediate-2 and 6 were high risk. In the univariate Cox analysis, degree of anemia (but not neutropenia and thrombocytopenia), IPSS, WPSS, revised cytogenetic risk stratification, a higher BM blast percentage (% BM blasts) as well as a higher number of total CD34+ cells and phenotypic abnormalities were associated with a shorter survival. In the multivariate analysis,only hemoglobin, % BM blasts and total CD34+ cells remained as independent risk factors. **Conclusions.** some variables generated by immunophenotyping of BM at diagnosis represent independent prognostic factors in MDS, and are important especially in a setting of patients with IPSS of low/intermediate risk and in cases with a normal karyotype. Supported by FAPESP, CNPq (INCTS) and MDS Foundation.

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HIPERFERRITINEMIA IN MDS PATIENTS- POLISH RETROSPECTIVE MDS REGISTRY Results

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Background. Most MDS patients have anemia and many of them require red blood cells (RBC) transfusions leading to iron overload. The most simple test assessing iron overload is serum ferritin concentration . **Aims.** Assessment of hyperferritinemia incidence in MDS patients at the moment of MDS diagnosis. Correlation between hiperferritinemia, RBC transfusions and clinical outcome in patients suffering from MDS. **Methods.** 196 patients (106 men and 90 women, mean age 72) out of 975 Polish MDS Registry patients were included into the study. Due to statistical correctness only patients registered within 3 months since diagnosis were included. **Results.** Serum ferritin levels were available for 105 patients and follow-up data for 78 patients. Ferritin level above 1000 ng/mL was found in 28 patients (27%) (Group 1) and ferritin level ≤1000 ng/mL in 77 patients (73%) (Group 2). Most patients with significant hiperferritinemia were RBC transfusion dependant (85% of patients). Among patients with ferritin level ≤1000 ng/mL 49% were RBC transfusion dependant. Serum hemoglobin concentration was lower in Group 1 patients in comparison with Group 2 patients (8 g/dL vs 9,2 g/dL, p< 0,001). The most frequent MDS subtype in Group 1 patients was refractory anaemia (RA) (28%) (in contrary to patients with ferritin ≤1000 ng/mL - 13%) (p<0,05). There were no differences between studied groups in IPSS score. Mean follow up was 8 months. There was a trend for longer overall survival (OS) in RBC transfusion independent patients (mean OS not reached) when compared to RBC transfusion dependant patients (mean OS 857 days, p=0,08). Mean OS was not significantly statistically different in studied groups (mean OS in Group 1 not reached; 2- 22 months in Group 2) p Log-Rank = 0,38. No correlation was found between ferritin level and time to AML transformation. **Conclusions.** Hiperferritinemia >1000 ng/mL does not influence survival and time to AML transformation in MDS patients. The most frequent MDS subtype in patients with ferritin level >1000 ng/mL was MDS RA. Among patients with ferritin level >1000 ng/mL 85% were RBC dependent.

1454

LDH =1. 5X ABOVE NORMAL IS A SENSITIVE AND SPECIFIC MARKER TO IDENTIFY PATIENTS AT RISK OF CLINICAL COMPLICATIONS AND MORTALITY ASSOCIATED WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a progressive disease caused by a somatic mutation in the *PIG-A* gene leading to deficiency of the complement protection proteins CD55 and CD59. Chronic, uncontrolled terminal complement activation leads to hemolysis, which is the underlying cause of the thromboembolism (TE), end organ damage, and early mortality associated with the disease. Lactate dehydrogenase (LDH) serum level of ≥1. 5 times the upper limit of normal (LDH ≥1. 5×) is a marker of uncontrolled complement activation. TE is the leading cause of death in PNH and accounts for 40%-67% of deaths in patients with PNH. **Aims.** To evaluate whether LDH ≥1. 5× at diagnosis is a suitable predictor of TE and mortality. **Methods.** This was a retrospective analysis of 224 patients with reported LDH levels at diagnosis

from the national PNH registry (N=301) in South Korea. **Results.** PNH patients with LDH ≥1. 5× at diagnosis had a 4. 8-fold greater mortality rate compared with an age- and gender-matched general population (AGMGP; *P*<0. 001), and in a multivariate analysis, LDH ≥1. 5× proved to be an independent predictor of mortality regardless of age, gender, or the presence of bone marrow disorder (OR=10. 57; 95% CI 1. 36-81. 93; *P*=0. 024). Patients with LDH <1. 5× had a similar mortality rate as the AGMGP (*P*=0. 824). A threshold LDH ≥1. 5× detected 93% of patient deaths and 96% of patients with TE. Attempting to potentially increase the specificity of identifying patients at risk of TE, an analysis was conducted with LDH threshold ≥3× or ≥5×. Neither LDH ≥3. 0× (OR 1. 8; 95% CI 0. 78-4. 09; *P*=0. 162) nor LDH ≥5. 0× (OR 2. 0; 95% CI 0. 91-4. 32; *P*=0. 082) were significant predictors of early mortality. Furthermore, LDH ≥3. 0× and ≥5. 0× only detected 67% and 47% of patients who experienced a TE, respectively. **Conclusions.** These data demonstrate that chronic uncontrolled complement activation as measured by LDH ≥1. 5× at PNH diagnosis is a strong and independent predictor of clinical complications and mortality in PNH patients. LDH ≥1. 5× clearly identifies PNH patients with a high risk of life-threatening complications and premature mortality (4. 8-fold) from the remaining population of PNH patients with normal survival. Thus, physicians should consider LDH ≥1. 5× as a strong indicator of risk for clinical complications and mortality that warrants early intervention.

1455

ELEVATED LDH IS ASSOCIATED WITH OCCURRENCE OF TE AND MORTALITY WITHIN 12 MONTHS OF ASSESSMENT IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder caused by a somatic mutation in the *PIG-A* gene that leads to chronic uncontrolled terminal complement activation. The subsequent red blood cell (RBC) hemolysis with platelet and leukocyte activation causes life-threatening thromboembolic events (TE), chronic renal insufficiency, pain, severe fatigue, poor quality of life, and early mortality. Lactate dehydrogenase (LDH) serum level ≥1. 5x the upper limit of normal (LDH ≥1. 5x) is a marker of uncontrolled complement activation that has been used in multinational PNH clinical trials and was recently confirmed by our group as a sensitive marker for measuring risk of mortality and TE in patients with PNH. **Aims.** To evaluate the association of LDH ≥1. 5x with risk of TE and mortality, and to understand the impact of granulocyte clone size on the occurrence of TE and mortality. **Methods.** A retrospective analysis of a 301-patient national PNH data registry in South Korea was performed. Patients were aged 8-88 years (median 37 years), median PNH duration was 6. 6 years (1 month-41 years), median PNH granulocyte clone size was 48. 8%, and median LDH was >4x above normal. 76. 3% of patients had an LDH ≥1. 5x and 18% (n=54) had a history of TE. **Results.** A significant association was shown between LDH and the occurrence of TE within 12 months of LDH assessment: the odds of experiencing a TE were 4. 4x greater if LDH was ≥1. 5x compared with <1. 5x (Table 1; *P*=0. 020). Importantly, 82% (18/22) of the patients with LDH ≥1. 5x who had a TE at 12 months experienced this TE within the first 6 months. Furthermore, 5% (7/150) of patients with LDH ≥1. 5x died within 12 months following LDH assessment, reflecting a significantly higher mortality compared with 0% (0/51) among the <1. 5x cohort ([*P*=0. 004], Table 1). In contrast, there was no correlation between clone size and risk of TE or early mortality. In a multivariate analysis across all interquartile clone size categories, there were no significant differences in the risk of TE and mortality (Chi square *P*=0. 292 and 0. 247, respectively). **Conclusions.** Elevated LDH is a significant predictor of increased occurrence of TE and mortality within 12 months of LDH assessment. It has been suggested previously that large clone sizes may predict risk of TEs; however, our findings from this large cohort of patients show that neither TE nor death within 12 months was related to clone size. The 15% (22/150) incidence of TE within 12 months in patients with LDH ≥1. 5, with most TEs occurring within the first 6 months of an LDH reading, highlights the medical need and urgency for early therapeutic intervention in PNH patients with an elevated LDH.

Table 1. Thromboembolism and mortality at 1 year after LDH assessment.

	P value	LDH <1.5x, % (n/N)	LDH ≥1.5x, % (n/N)
Thromboembolism	*P*=0.020	4% (2/53)	15% (22/150)
Mortality	*P*=0.004	0% (0/51)	5% (7/150)

1456

SAFETY AND EFFICACY OF 5-AZACYTIDINE IN MDS PATIENTS WITH MODERATE RENAL IMPAIRMENT: A SINGLE CENTER EXPERIENCE

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Background and Aims. The tolerability of 5-azacytidine allow its use in frail and/or elderly patients who comprise the majority of Myelodysplastic syndrome (MDS) population. Nevertheless, despite the increased incidence of renal impairment (RI) in older individuals, patients with RI were not included in the approval trials. As a result, the optimal use of 5-azacytidine in patients with RI is currently unknown. Only one single center, retrospective study addressed the efficacy and toxicity of 5-azacytidine in patients with RI, but no comparative analysis between patients with or without RI has been performed yet.

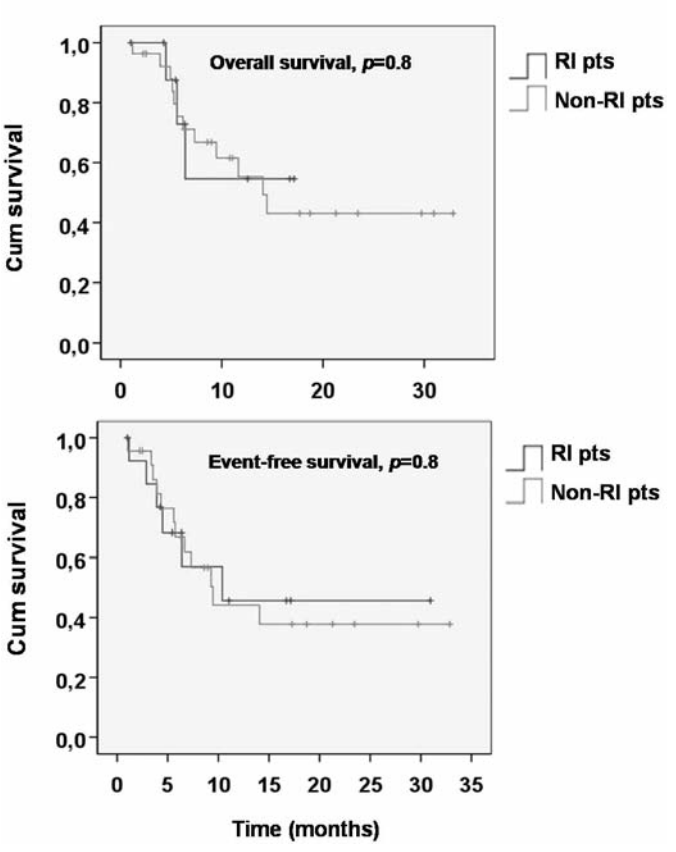

Figure 1.

Methods. We retrospectively analyzed 41 IPSS intermediate-2 and high-risk MDS patients (30 male, 11 female) with a median age of 73. 5 (range 55-81) years. Patients were classified according to WHO as RAEB-II (n=18), AML/MDS (n=5), CMML-II (n=8), MDS/MPN (n=4), RAEB-I (n=3) and RCMD (n=3). All patients had bilirubin level <2mg/dl, 11 (27%) had RI not requiring dialysis with median GFR 45 ml/min (range 22. 3-55, RI group) and 30 had median GFR 91 ml/min (73-140, non-RI group) as assessed by using the MRDM or the Cockroft-Gault formulas depending on patient's weight. Median follow up and number of completed cycles for all patients were 8. 6 months and 5(1-26), respectively. In all patients 5-azacytidine was started at 75mg/m2 SC for 7 days on 28-day cycles. Response to therapy and toxicity were evaluated using the IWG 2006 and NCI-CTC criteria, respectively. Significance of differences was assessed by Mann-Whitney U-test, Chi square or Fisher Exact tests as appropriate. Kaplan-Meier method and log-rank test were used for survival analysis. Event-free survival (EFS) was defined as the time from first 5-azacytidine administration to leukemic transformation or death. Results are presented as median values. **Results.** Patients with RI were significantly older than the non-RI ones (median age 76 vs 70, respectively, p=0. 004) and exhibited a trend for higher treatment discontinuation rate (54. 5% vs 23. 3%, respectively, p=0. 057). By contrast, no differences regarding gender, MDS subtype, transfusion needs and cytogenetics were noticed among the two groups. Likewise, patients with or without RI completed similar number of cycles (5 vs 7, respectively, p=0. 13) and displayed comparable rates of overall (not reached vs 14 months, p=0. 8) and event-free (10. 9 months vs 9,5 months, p=0. 8) survival (Figure 1), overall response (54. 5% vs 60. 7%, p=0. 9), dose adjustment (27. 3% vs 31%, p=0. 8), bleeding (45. 5% vs 38%, p=0. 8), infections (81% vs 72%, p=0. 73), days of hospitalization (14 vs 19. 5 p=0. 35) and grades 3-4 neutropenia (p=0. 14) and thrombocytopenia (p=0. 16). Renal function transiently deteriorated during the first 2 cycles, but then improved in 5/11 (45%) of patients with RI and 4/30 (13%) of non-RI group. Notably, adding the latter patients in the RI group did not alter the results of the comparisons. **Conclusions.** We conclude that 5-azacytidine is effective and safe in patients with moderate renal impairment who thus should be treated in a similar manner to patients with normal renal function. Transient decline in renal function may be observed in patients either with or without RI, but does not appear to affect the outcome.

1457

COMPASIVE USE OF 5-AZACITIDINE IN PATIENTS WITH LOW/INT-1 RISK MYELODYSPLASTIC SYNDROMES

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Background. 5-azacitidine (AZA) significantly prolonged overall survival in higher-risk patients with myelodysplastic syndromes (MDS) in international phase III trial (AZA-001). However, data about efficacy of AZA in lower risk MDS are less consistent and only few studies have addressed this topic. **Aims.** Evaluate efficacy and security of AZA in low/int-1 risk MDS patients. Materials and **Methods.** we evaluate the efficacy and safety in low and intermediate-1 risk MDS patients. **Results.** In our institution, a total of 36 patients were treated with AZA between 2006 to February 2012. We evaluated 21 patients diagnosed according to WHO criteria as low/intermediate-1 International Prognostic Scoring System (IPSS) risk MDS. At baseline, median age was 76 year old (range 46-86), male/female ratio 10/11. Median time from diagnosis to Aza treatment was 39 months (range 1-192). 80,9% patients were transfusion-dependent, 85,7% had received a prior treatment (rhu-EPO+G-CSF 44,4%, only rhu-EPO 55,5%). Low/Int-1 risk patients received AZA dose of 75mg/sqm/d subcutaneously during days 1-7 by 6 treatment cycles, and before 6th cycle they received same schedule by 5 days, in a 28-day cycle. The median number of monthly cycles was 12 (range 2-50), and 76,2% and 57,14 % completed at least 6 and 9 treatment cycles, respectively. 76,2% patients had a hematologic response, 4,8% patients have not response, and 19% patients had a stable disease. Grade 3-4 adverse events documented in these patients were neutropenia (9,5%), anaemia (4,7%) and thrombocytopenia (7. 4%). Non-hematological adverse events: injection site reaction 19%, 19% constipation, 19% diarrhea and 9% fever. Response duration ranged from 4 to 43 months (median 12 months). There were no significant differences in response rate according to age, previous treatment, transfusion requirements, basal EPO and Hb pre-AZA. 3 patients were transformed to AML after median 7,7 cycles of AZA (2-13). **Conclusions.** 1. - 76,2% patients achieved a hematologic response. 2. - Time to response is early (3months), although some patients response later (5 cycles or more). 3. - Efficacy and safety of AZA treatment is a valid alternative in low/int-1 risk MDS patients.

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HEMATOLOGICAL IMPROVEMENT DURING IRON CHELATION THERAPY IN MDS/IM PATIENTS: THE 'REL' (RETE EMATOLOGICA LOMBARDA) EXPERIENCE

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Background. Hematological improvement (HI) during iron chelation therapy (ICT) was first pointed out more than twenty years ago, both in Myelodysplastic Syndromes (MDS) and in Idiopathic Myelofibrosis (IM). This phenomenon seems to be more frequent after introduction of Deferasirox. However many questions about this topic need to be solved. **Aims.** To better understand frequency, duration and quality of HI during ICT. **Methods.** A multicentric retrospective data collection was conducted among 7 Lombard Hematological Centers afferent to "REL"(Rete Ematologica Lombarda") on MDS, MDS/MPD or IM patients treated with ICT for at least 6 months, up to 2 years of follow up. Transfusion requirement was defined as the number of RBC unit transfused in a three-month period. Data were tabulated and descriptive analysis was performed. IWG criteria of response published in 2000 (Cheson et al: Blood 96, 3671), was chosen instead of IWG 2006 criteria (Cheson et al: Blood 108, 419), because it was assumed that the possibility to split major and minor response was more appropriate in this model. All patients with a concomitant therapy that could influence HI were excluded from analysis. **Results.** On 53 patients, 49 were evaluable (45 MDS and 2 MDS/MPD and 2 IM). Median age was 67 years (range 24-27). Mean follow up was 18 months (range 6-24; median 24). All of them were transfusion dependent. Erythroid response was obtained in 15 patients (30. 6%): major in 4 (8. 1%) and minor in 11 (22%). Median time to response was 6 months (range 3-24). Median duration of response was 12 months (Figure 1). Notably none of the 4 patients who reached a major response showed a relapse before the end of observation period. Only 12 patients had platelet count less than 100. 000/mm^3. Of them 7 (58. 3%) had platelet response (6 major and 1 minor response). Median time to response was 5 months (range 1-14). In only one case there was the loss of response after 4 months. In the other cases response was maintained after 1 (2 cases), 8, 12 and 24 (2 cases) months. Only 16 patients were neutropenic (neuthrophils less than 1500/mm^3). Ten of them (62. 5%) showed a major response. Median time to response was 3. 5 months (range 1-16). In two cases response was lost after 1 and 3 months. In 4 cases response was maintained after 1, 10 and 21 (2 cases) months. In the other 4 cases response was lost and successively recovered, and still ongoing at the end of follow up. In 23 of the 49 patients (46. 9%) at least a minor response of some kind was seen. In only one case there was a tri-linear response. Bi-linear response was seen in 7 cases, all of them with erythroid response. In the remaining 7 cases of erythroid response only one patient had a further, not responsive, cytopenia (thrombocytopenia). **Conclusions.** HI after ICT seems to be more frequent than expected. In a not negligible number of cases HI is clinically relevant. Clinical and biological features than can influence HI need to be investigated.

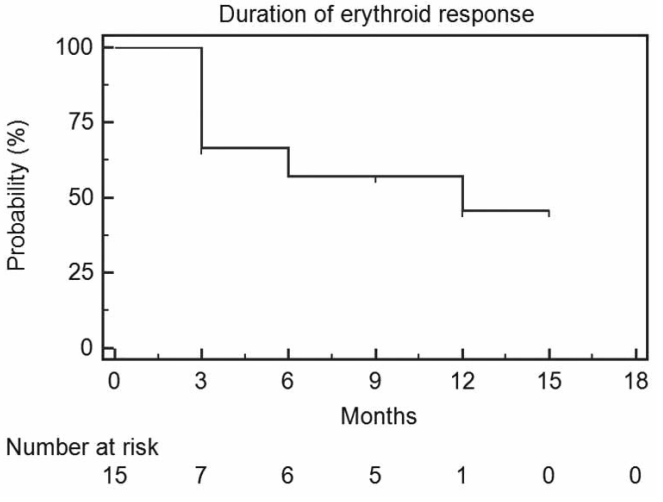

Figure 1.

1459

ANALYSIS OF SHORT-TERM EFFICACY OF LENALIDOMIDE IN PATIENTS WITH INTERMEDIATE-1 RISK MYELODYSPLASTIC SYNDROME WITHOUT 5Q DELETION

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Objectives To evaluate the efficacy of lenalidomide in the treatment of the intermediate-1 risk non-del□5q□myelodysplastic syndrome□MDS□. **Methods** 30 patients were diagnosed as MDS by the peripheral blood count, bone marrow morphology and Cytogenetic analysis. There were 14 males and 16 females. the median age was 52 year- old (13 ~ 83). The average time from diagnosis to treatment was 4. 1 (0~9) months. cytogenetic analysis: All patients were classified by G-banding chromosome karyotype analysis and fluorescence in situ hybridization (FISH). We chose combined probes of +8, -5/5q-, -7/7q-, 20q-,-Y, -12/12p- in FISH detection. According to the WHO classification system, 6 patients were classified as RA□1 was RN□2 were RT□1 was RARS, 15 were RCMD□5 were MDS-U. 7 cases with 20q-,2 cases with +8□1 case with dup(1) (q22□31) and 4 cases were failed in karyotype analysis. Meanwhile, we found 4 cases with +8□7 cases with 20q-□3 cases with 12p-□16 cases with negative results in FISH test. According to the International Prognostic Scoring Systems (IPSS), 21cases got a score of 0. 5, 9 cases got a score of 1. 0 . we Revilmid 10 mg/d for 21 days every 28 day. All 30 cases were treated with lenalidomide 3 cycles at least, 20 of them received 4 cycles. **Results.**Before treatment, absolute neutrophil was 0. 95±0. 47×10^9/L, hemoglobin was 59. 1±16. 06 g/L, platelet was 30. 7±67. 8×10^9/L. After 2 cycles□30 cases□ ,absolute neutrophil was 1. 02±0. 59×10^9/L, hemoglobin was 61. 6±16. 8 g/L, platelet was 40. 2±18. 8×10^9/L. After 4 cycles□20 cases□ absolute neutrophil was 1. 28±0. 84×10^9/L, hemoglobin was 68. 6±14. 8g/L, platelet was 50. 1±85. 6×10^9/L. Lenalidomide had been shown the efficacy in patients with the Int-1 risk non-5q- MDS. Peripheral blood cell counts were improved after treatment 2 to 4 cycles. The Changes in Peripheral blood cell counts between the patients with 0. 5 scores less than 3 months from diagnosis to treatment and the patients with 1. 0 scores more than 3 months from diagnosis to treatment was statistically significant (P□0. 05). Platelet recovery in the former was faster and more obvious, platelet recovery was the fastest in the patients with 20q-. All patients got stable disease after 3 cycles, 14 cases with chromosome abnormalities by FISH test were found no cytogenetic response to the treatment. **Conclusions** Patients with the intermediate-1-risk non-del□5q□MDS treated with lenalidomide had not achieved complete hematologic remission, but could get hematologic improvement. At least, most patients needn't platelet transfusion and keep a safe platelet level without spontaneous bleeding□≥30×10^9/L□,at the same time, most patients achieved Erythrocytes transfusion independence (≥60g/L) after treatment.

1460

PNH CLONAL EXPANSION FOLLOWING STEM CELL TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA: A RETROSPECTIVE ANALYSIS IN A BRAZILIAN HOSPITAL

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a chronic and life-threatening hematopoietic stem cell disorder characterized by deficiency of the GPI-anchored complement inhibitory proteins CD55 and CD59 on blood cells. The resulting uncontrolled complement activation is responsible for chronic hemolysis and can lead to serious clinical morbidities including thromboembolism (TE) and chronic kidney disease (CKD), which have been shown to increase risk for mortality. While stem cell transplantation (SCT) remains the only potentially curative option for PNH, the risk for substantial morbidities and mortality still exist. In patients with PNH, up to 45% of patients receiving bone marrow transplantation die or develop graft-versus-host disease (GVHD); however, in patients with severe aplastic anemia (SAA), this may be the only option to address severe pancytopenia if immunosuppression therapy does not work. **Aims.** Presentation of case series demonstrating the appearance of PNH clones in SAA patients post-SCT. **Methods.** This study retrospectively analyzed 519 SAA patients who underwent SCT for severe pancytopenias between 1979 and 2012. SAA was defined by using the Camitta criteria, with marrow cellularity <25% overall or <50% with <30% hematopoiesis, and two of the following: neutrophil count less than 500/μL, anemia with corrected reticulocyte count <1%, or platelet count <20,000/μL. **Results.** Six patients (1. 2%) developed clinical and laboratory characteristics of PNH which were not present prior to SCT. At the time of SCT these patients, half of whom were female, median and range for select demographic and clinical characteristics were: age 21 years (17-38 years); absolute neutrophil count per μL 224 (88-576), platelet count per μL 6,000 (4,000-16,000), and number of transfusions before SCT 15 (4-45). All 6 patients were transplanted with bone marrow stem cells from HLA-identical siblings following administration of preparatory cyclophosphamide (200 mg/kg) and GVHD prophylaxis, including use of methotrexate plus cyclosporine. One patient developed grade II acute GVHD. PNH was confirmed by positive Ham/sucrose test in 2 patients and by flow cytometry in 4 patients (Table 1). The median time from SCT to clonal expansion was 101 months (range: 51-182 months). Two patients underwent a repeated SCT using the same donor and same GVHD prophylaxis, but using a preparatory regimen containing busulfan (8 mg/kg) plus fludarabine (125 mg/kg). As of January 2012, 1 patient had died due to severe pancytopenia 7 years after SCT; the remaining 5 patients are alive with no evidence of severe cytopenias. **Conclusions.** This is the first case series from our hospital in Brazil detailing the development and expansion of PNH clones following SCT in patients with severe pancytopenias. These findings demonstrate that while SCT is appropriate for SAA patients, it may lead to PNH clonal expansion in some patients. This case series demonstrates that it is important to monitor patients post-SCT for signs and symptoms of PNH. In light of recent data presented on prolonged survival and enhanced patient outcomes associated with eculizumab, these patients may be ideal candidates for this treatment choice when made available in Brazil.

Table 1. Percent deficient granulocytes and monocytes in 4 patients diagnosed with PNH using flow cytometry.

	Patient				Median
	1	2	3	4	
% deficient granulocytes	1.6	63.0	97.0	98.0	80.0
% deficient monocytes	2.5	77.5	99.6	99.9	88.6

1461

AZACYTIDINE 75MG/M2 X5 DAY IN MYELODISPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA REFRACTORY/RELAPSED PATIENTS: Results FROM A SINGLE CENTRE

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Purpose. To analyze the results of administration Azacytine (75mg/m^2x5 days) in myelodisplastic syndrome (MDS) patients and acute myeloid leukaemia refractory/relapsed (AML) on an outpatient basis. Each cycle was performed every 28 days at a dose of 75 mg/m^2X 5 days. Patients and schedule: 46 patients diagnosed of MDS or AML (September 2007-November 2010). We treat 25 patients with the diagnosis of MDS. IPSS was high in 3 (12%), intermediate II in 13 (52%), intermediate I in 8 (32%) and low in 1 patient. AML was the diagnosis of 21 patients. AML refractory to therapy were 3 (15%), secondary to MDS or myeloproliferative disease in 7 (35%) and AML de novo in older or not fit in 10 (50%). WHO categories were: AREB-1: 3; AREB-2: 3; RCMD:3; MDS-U: 2; LMM-2: 3; AML refactory or relapsed: 8; AML secondary to MDS: 10. 17 patients have secondary AML or SMD (1: after aplastic anemia; 1: prostate carcinoma treated with radiotherapy; 1: Hodgkin disease and autologous stem cell transplantation; 1: mastocytosis plus chronic lymphocytic leukema; 1: chronic Neutropenia; 1: rectal carcinoma; 1: Acute Promyelocytic leukemia, LMA-M5 trasplanted 3 years before and with a new -7q, 1: multiple myeloma; 1 polycytemia vera, 1: myelofibrosis; 1: renal carcinoma; 1: essential thrombocytemia). Cytogenetic risk: good: 24 patients; intermediate: 4 patients, high risk: 17 . Hydrea or thioguanine was permitted to control leukocyte counts in AML patients. Azacytidine was prescribed x 5 consecutive days (Monday to Friday) in an outpatient basis. Response criteria were evaluated each cycle and at 6th cycle by blood count and bone marrow aspiration (defined by IWG 2006). Non-responders patients were withdrawn of the treatment after 6th cycle. In cases of bone marrow response but cytopenia, the courses were delayed after the sixth cycle. **Results.** The median age of the 46 patients was 74,3 year (36,1-87,6). (Male/Women: 32/9). The median time to treatment was 1,8 months (0,0-38,53). The median number of cycles administrated was 7,5 (1-32). Response to treatment and survival: Bone marrow CR +PR at 6 months was 17 (38. 7%)(CR: 10; PR: 6). Transfusion independence, platelet and neutrophil responses were obtained in 17 patients. Median cycle to obtain response was the third cycles (1st- 7th). No differences in response were obtained

between MDS and AML. Median survival of all the patients was 13. 677. The median survival of AML group was 9,2 months; the median survival of MDS was 14,997 (p<0,3146) (the same or better than reported by Itzykson, MDS French group. Two AML patients obtained complete response). This trial was not comparative, but 5 days schedule seems as effective as 7 days treatment in very unfavourable prognostic patients.

References

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1462

OUTCOMES OF SALVAGE THERAPY FOR PATIENTS WHO RELAPSED AFTER AZACITIDINE TREATMENT WAS POOR REGARDLESS OF INTENSIVE THERAPY

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Background. Hypomethylating agents improved clinical outcomes of myelodysplastic syndrome (MDS) patients. However, the treatment outcomes of patients who relapsed after azacitidine treatment has not been determined yet. **Aims.** The current study evaluated outcomes of salvage therapies for MDS patients who relapsed after azacitidine treatment. **Methods.** The outcomes of salvage treatment of 49 MDS patients who relapsed after previous azacitidine treatment were evaluated in the current study.

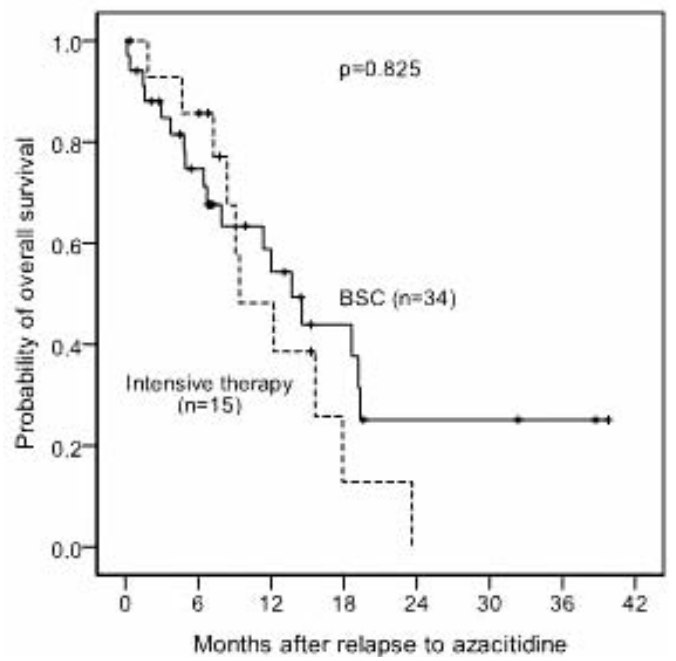

Figure 1. Survival rates after relapse to azacitidine treatment.

Results. Azacitidine was administered median 8 cycles (range 4-20 cycles) prior to relapse. Thirty-four patients were received best supportive care (BSC) after relapse to azacitidine and 15 patients were treated with salvage therapy; intensive chemotherapy for 6 patients, allogeneic stem cell transplantation for 2 patients and other hypomethylating therapy (decitabine) for 7 patients. Survival duration of relapsed patients after azacitidine was median 367 days (range 248-485 days). For BSC group, survival duration was median 367 days (range 248-485 days) with 1-year OS rate of 54. 3+9. 7% and for salvage therapy group, survival duration was median 282 days (range 136-427 days) with 1-year OS rate of 48. 2+15. 1% (p=0. 825). When considered to IPSS risk groups, the salvage therapies were not effective compared to BSC. Of the 31 IPSS low and intermediate-1 group, survival duration was median 436 days (range 298-573 days). One-year OS rate was 59. 9+11. 2% (BSC) and 54. 7+20. 1% (salvage therapy) (p=0. 977). Of the 18 IPSS intermediate-2 and high group, survival duration was median 272 days (range 215-328 days). One-year OS rate was 42. 1+17. 7% (BSC) and 41. 7+22. 2% (salvage therapy) (p=0. 473). **Conclusions.** Although azacitidine improved survival rates in MDS patients, outcomes of salvage therapy for patients who relapsed after azacitidine treatment was poor regardless of salvage therapy compared to BSC. So, active incorporation of intensive therapy to modify the disease course would be needed.

1463

HOME AZACITIDINE (AZA) ADMINISTRATION IN MYELODYSPLASTIC SYNDROMES, CMML AND ACUTE MYELOID LEUKEMIA

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Background. AZA 75 mg/m^2/day x 7 days is the treatment of choice for high-risk MDS and a therapeutic option for AML in old patients. Prescribing recommendations insist in the administration of the drug by trained nurses or doctors. Due to the fact that our Outpatient Clinic remains closed on weekends, the compliance with the standard dosage of 7 consecutive days has been a difficult issue. Therefore, we devised a system in which the nurse-in-charge instructed relatives on the preservation and administration of AZA. Our hypothesis is that this way modality does not affect efficacy or tolerance. **Aims.** Trying the feasibility of 2 home-administered doses of AZA after 5 doses in the Outpatient Clinic, as a way of complying with the 7-day AZA regimen. **Methods.** The Hospital Pharmacy staff reconstituted the vial with 4 ml of sterile water for injection and distributed it in two syringes of 2 ml each. Both were delivered to the relative every day in an isothermal package and then administered to the patient without any other manipulation, subcutaneously, within a period of 8 hours. The relative willing to collaborate was trained from the first cycle, regarding the way of administration, the prevention of local side effects and the importance of preserving the environment of the drug. The nurse-in-charge finally assessed the skills and understanding before the final approval. Since this strategy implied a deviation from the rule, an informed consent was obtained for every couple patient/ relative, as well as an explicit authorization from the physician. **Result.** From August-2009, 23 patients (15 M / 7 F) were included in this home chemotherapy program. The mean age was 71. 25 yr. (range 41- 82). Diagnoses according to the WHO-2008 were AML (10), RCMD (4), AREB1 (3), AREB2 (2), AR / ARS (3), CMML (1); risk stratification according to IPSS for MDS were: high (1), INT-2 (6), INT-1 (4), 2 non applicable. A total of 149 cycles were administered with this modality (1-21 cycles per patient); no immediate complications led to hospital admission. No missing doses, failed administration or transgression in the preserving conditions were recorded. The rate of local reactions requiring intervention was similar to those reported with the standard regimen. Best recorded response after 4 - 9 cycles was:- AML: CR (1), PR (4), failure (4); 1 patient died after the 1st cycle. - MDS (IWG-2006 criteria): 4 not assessable (less than 4 cycles, due to early death, discontinuation or recent start of AZA); 2 failure; 1 stable disease, 4 partial response, 1 medullar response; 1 proceeded to allo-BMT. Hematological improvement was not considered. Further data and updating of the former would be provided on June-2012. **Summary and Conclusions.** Home administration of Azacitidine by relatives was safe and feasible, allowing the 7-day scheme when no facility is available on weekends. The core factors for the success of this strategy were: informed consent; willingness of relatives to participate; systematic education; collaborative Hospital Pharmacy.

1464

CRITICAL ANALYSIS AND VALIDATION OF THE AZACITIDIN PROGNOSTIC SCORING SYSTEM (APSS)

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Background. Azacitidin (AZA) significantly improves survival in higher-risk MDS patients compared to conventional treatments (AZA001 trial). With response rates of about 50% predictive factors are of major interest. Recently a AZA prognostic scoring system (APSS) was proposed (Itzykson,Blood 2011), considering ECOG performance status (≥2=1point), circulating blasts (present=1pt), RBC transfusion dependence (≥4 U/8wk=1pt) and the IPSS cytoge-

netics (good=0, int=1 and poor=2 pt) as variables and defining three significantly different prognostic subgroups: low (score 0), intermediate (1-3) and high risk (4-5) In a retrospective single center analysis we tried to validate this interesting tool. **Results.** Between 03/2009 and 04/2011 34 patients (21 males,13 females) with high risk MDS or AML evolving from MDS or with dysplastic features received AZA until progression. At time of the analysis, 20 pts are alive, nine of them still on treatment (total of 227 cycles). The median age at time of diagnosis was 72 years (range 48-90). 17 patients received AZA from diagnosis, the median time from diagnosis to treatment for the others was 7mo (range 1-54). The pts received in median 15 cycles (range 1-21) and, with 31 pts. evaluable, remission rates were: CR 22%, PR 10%, stable disease 22,5%, non-responder 45,5%. The med. survival duration was 37 months from time of diagnosis and 14,5 months from AZA onset. Focusing on the APSS risk factors, ECOG PS ≥2, RBC transfusion ≥4 units/8 weeks and circ. blasts were present in 44%, 71% and 59% of pts respectively. Cytogenetics were good, intermediate, and poor in 70%, 18% and 12%. 4 pts. (12%) were considered low, 24 (73%) intermediate and 5 (15%) high risk, being very good comparable to the distribution of the APSS (11%, 71% and 18% resp.). With a median f/u of 21,4 months (from diagnosis), 12,3 months (from start of AZA), median OS from time of diagnosis were 72, 37 and 7 months in patients with low, intermediate, and high risk score respectively. Accounting for the time of AZA treatment initiation survival data were 18, 13. 5 and 6 months respectively and the number of AZA cycles adminstered were 9, 8 and 4 respectively. **Conclusions.** The results of this single center analysis are in agreement with the AZA prognostic scoring system based on conventional prognostic factors. As in our opinion the specific predictive value of the APSS regarding response to AZA treatment remains unclear, comparative data of untreated higher risk patients will be presented at the meeting.

1465

IMPROVEMENT OF QUALITY OF LIFE IN PATIENTS WITH REFRACTORY ANEMIA WITH EXCESS OF BLASTS: ROLE OF 5'-AZACYTIDINE COMPARED WITH CONVENTIONAL CARE REGIMENS. A SINGLE CENTRE STUDY

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Background. 5'-azacytidine (5-AZA) is the current standard treatment for high-risk myelodysplastic syndrome, increasing overall survival in relation to conventional regimen (Fenaux,2010). **Aims.** We want compare quality of life, transfusion and hospitalization rate of patients with refractory anemia with excess of blasts (RAEB), observed in our division, before and after introduction of therapy with 5-AZA. **Methods.** Between 2006 and 2011 we observed 30 cases of RAEB (4 was RAEB type I and 26 RAEB type II); median age 72. 5 years (range 60-84 years). 19 received conventional care regimens (10 received only best supportive care, 5 low dose chemotherapy, 4 intensive chemotherapy), because 5-AZA wasn't yet available in our institution; 11 received 5-AZA at a dose of 75 mg/m2 per day for 7 days every 21 days. This therapy was given for a median of nine cycles (range 4-24 cycles). **Results.** For conventional care regimen group, median survival was 9 months (range 2-23 months); acute myeloid leukemia (AML) transformation occurred in 13 patients (68%) and median time to evolution was 4 months (range 1-20 months). In addition, there were frequent hospitalizations mainly for infectious causes, with an average of 3 days of hospitalization per month for patient. The mean monthly transfusional rate was 1. 9 units of packed blood red cells and 3. 9 units of platelets concentrates. In 5-AZA group, 4 patients died for other causes after a median of 4 cycles (range 3-10 cycles) with a median survival of 11 months (range 3-31 months). 7 patients are currently in therapy with 5-AZA with a median follow-up of 9 months (range 4-29 months). No evolution in AML was showed. The mean monthly hospitalization rate was 0. 6 days for every patient. The mean monthly transfusional rate was 1. 1 units of packed blood red cells and 0. 5 units of platelet concentrates. 8 patients (72%) of 5-AZA group was transfusion dependent at diagnosis. Five of this patients reached transfusion independence after a median of 3 cycles (range 2-7 cycles). *Discussion.* 5-AZA group showed a better quality of life compared with conventional care regimen group. Indeed, analysis of data reported above shows: 1) a clear reduction of hospital days (reduced to one-fifth), 2) a reduction of transfusional rate and early achievement of transfusion independence (in about 60% of transfusion-dependent patients), 3) improvement of performance status. **Conclusions.** Treatment with 5'-azacytidine improves quality of life in patients with refractory anemia with excess of blasts.

1466

CLINICAL FEATURES CONDITIONING HEMATOLOGICAL IMPROVMENT DURING IRON CHELATION THERAPY IN MDS/IM PATIENTS. THE 'REL' (RETE EMATOLOGICA LOMBARDA) EXPERIENCE

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Background. The possibility to have an hematological improvement (HI) during iron chelation therapy (ICT) has been described, but clinical or biological features than may be associated to this phenomenon are still unknown. **Aims.** To verify if some clinical features, detected before beginning of ICT, may influence HI. **Methods.** A multicentric retrospective data collection was conducted among 7 Lombard Hematological Centers afferent to "REL"(Rete Ematologica Lombarda") on patients with myelodysplastic syndrome (MDS), myelodysplastic/myeloprolifertive disease (MDS/MPD) or Idiopathic Myelofibrosis (IM). All patients were transfusion dependent and treated with ICT for at least 6 months, up to 2 years of follow up. According to IWG criteria of response published in 2000 (Cheson et al: Blood 96, 3671), were considered as responsive patients those with at least a mono-linear minor response in at least one of the three lineages. Some clinical features referring to the moment of the beginning of ICT were tabulated and analyzed with the usual descriptive methods. Comparison between categorical variables were carried out by Fisher's exact test; the logistic regression with one or two independent regressors was used to verify the possible existence of association between HI and categorical or continuous variables. **Results.** On 53 patients, 49 (45 MDS and 2 MDS/MPD and 2 IM) were evaluable. Of them 31 were male, and median age was 67 years (range 24-27). Mean follow up was 18 months (range 6-24; median 24). In 23 of them (46. 9%) we detected an HI as defined above. A possible correlation with the response was analized for the following variables: gender; age; transfusional burden (number of RBC unit in the three previous months); presence of neutropenia (<1500/mm^3); presence of thrombocytopenia (<100. 000/mm^3); iron chelator (deferasirox or deferoxiamine); seric iron, transferrin, ferritin, lactatedeidrogenase (LDH), epoetin; response to iron chelation (reduction of ferritin levels above 35% after 12 months). For MDS patients only, also the following were included in the analysis: diagnosis according to FAB and WHO, cytogenetic risk according to IPSS stratification, presence of bone marrow fibrosis and percentage of bone marrow cellularity. Univariate analysis shows that only the presence of neutropenia influences significantly HI (p< 0. 005). Among other parameters only LDH and epoetin seems to have ability to influence response, but they do not reach a statistical significance (respectively: p = 0. 06 and p = 0. 07; odds ratio: 0. 4% and 0. 2%). Bi-variate analysis were also performed considering neutropenia with LDH and neutropenia with epoetin as independent potential regressors: the presence of neutropenia maintained a predictive value on response in both models (respectively p =0. 003 and p = 0. 001). **Conclusions.** in this series the presence of neutropenia enhances the probability of HI during ICT. No other clinical predictive factor has been detected. This findings need to be confirmed in a larger and prospective court. Biological studies to identify patients with good chance to ameliorate cytopenias under ICT are also warranted.

1467

SECONDARY HEMATOLOGIC MALIGNANCIES IN PREVIOUSLY-TREATED PATIENTS WITH SOLID TUMOR

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Background. Secondary hematologic malignances may occur as a late complication following various cytotoxic therapy, including chemotherapy, radiotherapy, or radioactive iodine therapy. **Patients and Methods.** We retrospectively analyzed 346 patients referred to our hospital for newly diagnosed leukemia or multiple myeloma from January 2000 to December 2009. There were 23 patients with a past history of cytotoxic therapy for solid tumor. **Results.** The median age was 61 years (range, 29-75). The median interval between secondary hematologic malignancy development after beginning cytotoxic therapy for various solid tumor was 33 months (range, 6. 6-59. 9). Solid tumors include breast cancer (n=7), thyroid cancer (n=4), cervix cancer (n=3), lung can-

cer (n=3), colorectal cancer (n=2), nasopharyngeal cancer (n=1), hepatocellular carcinoma (n=1), osteosarcoma (n=1), and pheochromocytoma (n=1). Secondary hematologic malignancy include acute myeloid leukemia (n=6), myelodysplastic syndromes (n=6), chronic myelogenous leukemia (CML) (n=5), multiple myeloma (MM) (n=3), acute lymphoblastic leukemia (n=1), and biphenotypic acute leukemia (n=1). Treatment modalities include chemotherapy alone (n=11, 47. 8%), radiation (RT) along (n=4, 17. 4%), both chemotherapy and RT (n=3, 13%), 131-iodine therapy (n=4, 17. 4%) and iodine-l31 metaiodobenzylguanidine (n=1, 3. 4%). Patients had received various cytotoxic agents, including alkylating agents (8 patients, 34. 8%) and topoisomerase 2 inhibitors (8 patients, 34. 8 %). In patients who received RT, one developed acute leukemia, 4 developed CML and 2 developed MM. Interestingly, 3 patients who received intracavitary RT for cervical cancer developed CML (n=2) or MM (n=1). **Conclusions.** The risk of secondary hematologic malignancies as a late complication following various cytotoxic therapies must be kept in mind and close monitoring and follow-up would be required.

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AZACITIDINE IN CMML, REPORT OF THE SPANISH EXPERIENCE.

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Background. Treatment of CMML remains a clinical challenge, with no drug demonstrating clear clinical benefit. AZA yielded a survival benefit in higher risk MDS and in a few patients with CMML and have been aproved for its use. **Methods.** A cohort of CMML pts (according to WHO 2008 classification) treated with AZA in Spain were reviewed. **Results.** Between 2006 and 2011, 36 CMML pts received AZA. The median age was 70 y (range 33-90), M 74%/F 26%. These registry includes 21 patients (58%) with CMML-1 and 15 patients (42%) with CMML-2. Myeloproliferative CMML (MP-CMML), was present in 18/36 patients, whereas 18/36 had myelodysplastic CMML (MD-CMML). Thus, the registry includes patients for whom AZA is currently not approved by the FDA/EMEA 25/36. Prior to AZA: palpable SM were present in 21% of patients, 60% of patients were in trasfusion dependence and 19% pts had received intensive chemotherapy. In the 29 patients in whom pretreatment cytogenetics were performed, 21, 5 and 3 could be grouped into IPSS good, intermediate and poor risk categories, respectively. The median number of cycles of AZA administered was 6 (range 1-24). The median OS was 1. 17 months. Detailed response and OS statistical analyses of various factors known or thought to influence prognosis are currently being analyzed, including MP-CMML vs. MD-CMML, CMML-1 vs. CMML-2, comparison of IPSS cytogenetic risk groups, as well as treatment-naïve vs. pretreated patients. In conclusion, in this population of partly pretreated, old patients with CMML, AZA was well tolerated and yielded substantial clinical and hematological benefit.

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CLINICAL ANALYSIS OF 18 PATIENTS WITH MYELODYSPLASTIC SYNDROME-REFRACTORY ANEMIA WITH EXCESS BLASTS(MDS-RAEB) TREATED WITH DECITABINE

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Objective. To summarize the recent therapeutic effect and adverse reaction of treatment with decitabine in myelodysplastic syndrome-refractory anemia with excess blasts(MDS-RAEB). **Methods.** 18 patients with MDS-RAEB treated in our cancer centre from September 2009 to December 2011 were analyzed retrospectively□including 10 men and 8 women□ranged from 28 to 72 years old □median age was 49 years,11 patients were diagnosed as MDS-RAEB-2□7 patients were diagnosed as MDS-RAEB-1; Chromosome karyotype analysis: 6 patients were favorable karyotype, 6 patients were intermediate karyotype, 4 patients were unfavorable karyotype and the other 2 patients were unsuccessful to karyotype analysis. According to the IPSS risk criteria, 2 patients belonged to intermediate-1 risk group□14 patients belonged to intermediate-2 risk group, 2 patients belonged to high risk group. All patients were treated with decitabine dosing 20 mg/m^2/day for 5 days per cycle□the intermission of each cycle ranged from 4 to 6 weeks. 5 patients received 1 cycle, 8 patients received 2 cycles□4 patients received 4 cycles□1 patient received 6 cycles. **Results.** 3 patients achieved Complete remission (CR),among them, 1 patient achieved CR after 2 cycles of decitabine, the other 2 patients got CR after 3 cycles of decitabine. The chromosome karyotypes of the 3 patients were 20q-□The remission duration was longer than 18 months. 2 patients achieved molecule remission(FISH was negative) after 18 months. The CR patients received 4 cycles of decitabine treatment at least; 2 patients achieved partial remission (PR), 5 patients attained hematology improvement□HI□. on the whole□12 patients got benefit from the treatment with decitabine□including CR/PR/HI□ the overall response rate was 55. 6%. 4 patients stopped therapy because they progressed to acute leukemia during the treatment with decitabine. 1 patient died of the complication of septicemia and septic shock. The main adverse reaction was hematotoxicity, especially significantly after 1or 2 cycles of treatment□the absolute neutrophil count of 3 patients reduced to 0, 6 patients' reduced to below 0. 1×10^9, the period of agranulocytosis maintained 1-4 weeks □9 patients were infected after 1-2 cycles of the treatment□5 of them had serious infection□for example□perianal abscess, septicaemia, Pneumonia of fungal infection. the patients infected seriously were older patients and had the complications of chronic disease, for example, diabetes, chronic obstructive pulmonary disease□and long history of MDS. The hematotoxicity became mild after 4-6 cycles of the treatment, no cumulative toxicity, no hepatotoxicity or nephrotoxicity. **Conclusions.** The response rate of recent therapeutic effect of treatment with decitabine in MDS-RAEB achieved above 50%□ the main adverse reaction was hematotoxicity. **Key words.** MDS□Treatment; Decitabine

1470

SURVEY-BASED HEMATOLOGIST VIEW OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: AN OPPORTUNITY FOR IMPROVEMENT OF DIAGNOSIS AND MANAGEMENT

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a genetic, progressive, life-threatening disorder characterized by chronic, uncontrolled terminal complement activation and hemolysis. The disease has a prevalence of 2. 0-15. 9 cases/million and a median age of onset of 33 years. Early diagnosis and intervention is critical; however, PNH is associated with diverse symptoms that often make diagnosis difficult to establish (average delay in diagnosis is 2. 5-3 years). 35% of patients with PNH die within 5 years of diagnosis. **Aims.** To assess clinicians' awareness of PNH, identify causes of delay in PNH diagnosis, determine the signs and symptoms that prompt clinicians to test for PNH, and gain insight into how PNH is managed in different regions. **Methods.** A survey comprising 43 questions was distributed to hematologists involved in the management of PNH throughout the Middle-East, North Africa, and Central and Eastern Europe. **Results.** Of the 70 physicians who completed the questionnaire, 90% were hematologists, with 33% specializing in hematologic oncology. Responders were familiar with PNH (91%) and with syndromes associated with PNH (myelodysplastic syndromes [97%]; aplastic anemia [96%]). 59% were currently managing ≥1 patient with PNH and 64% had diagnosed the disease within the past 3 years. Median time from clinical presentation to diagnosis was 6-9 months (range 3 months to 3 years). In general, patients with symptoms indicative of PNH were tested for the disease: 74% of patients with aplastic anemia, 42% with myelodysplastic syndrome, 74% with hemoglobinuria, 53% with unexplained thrombosis, 59% with Coombs-negative hemolytic anemia, and 47% with unexplained cytopenia. Almost all responders (98%) used flow cytometry to confirm diagnosis of PNH and the majority (77%) were aware that both erythrocytes and granulocytes or monocytes should be tested by flow cytometry to confirm diagnosis. Of the palliative medications prescribed to patients with PNH, blood transfusion was the most common (90% of patients), followed by immunosuppressants (48%) and iron supplements (42%). Almost all responders (98%) were aware of eculizumab; however, only 28% of patients were prescribed eculizumab. 17. 5% of patients underwent bone marrow transplantation (Middle-East and North Africa, 25%; Central and Eastern Europe, 10%). **Conclusions.** Within a population of hematologists with experience of

PNH, diagnosis may still take up to 3 years. The use of flow cytometry, the 'gold standard' for PNH diagnosis, is widespread, though greater awareness of the symptoms indicative of PNH is required. Palliative medications (immunosuppressants and iron supplements) are widely used despite having no impact on PNH[1]. Bone marrow transplantation is still used, particularly in the Middle-East and North Africa. This is despite its risk for substantial morbidities and mortality, and despite being indicated only for those patients with signs of bone marrow failure[2]. In spite of increased awareness of eculizumab effectiveness and its ability to reduce hemolysis and normalize survival[3], its use is not yet widespread in these regions. *Survey funded by Alexion. Authors received no financial support.*

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IMPACT OF COMORBIDITY ON TREATMENT WITH 5-AZACITIDINE AND OUTCOME IN 39 PATIENTS WITH MYELODYSPLASTIC SYNDROME: A SINGLE CENTER POPULATION ANALYSIS

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Background. Myelodysplastic syndromes (MDS) are a heterogeneous group of neoplastic disorders, with variable clinical course, originated from a clonal disorder of hematopoietic cell. MDS affects most frequently old individuals, who usually have an increased prevalence of comorbidities. Several studies are evaluating the impact of comorbidity on the natural history of the disease and on treatment decisions. The introduction of new hypomethylating agents, such as 5-azacitidine (AZA), extended the range of potentially curable people. **Aims.** To assess the impact of comorbidities, present at diagnosis, on the management and efficacy of therapy with AZA in patients with myelodysplastic syndrome. **Methods.** A retrospective analysis was conducted on 39 MDS patients admitted to our institution and treated with AZA. The diagnosis was established according to the 2008 WHO criteria; IPSS and WPSS prognostic scores were calculated. Comorbidities were defined according to three scales: CCI (Charlson comorbidity index), HCT-CI (hematopoietic cell transplantation-specific comorbidity index) and MDS-CI (myelodysplastic syndrome comorbidity index). The response to treatment with AZA was defined according to IWG 2006 criteria. **Results.** The mean age at diagnosis was 68 years and almost half of the patients was older than 70 years. The mean number of cycles was 6,13 (range 1-20); 51,3% performed less than 6 cycles. According to the three scales of comorbidity, on average 25% of patients had a high risk score. Comorbidity was not significantly correlated neither with a worst outcome nor with a less number of administered cycles. The overall response rate (complete response, partial response and hematological improvement) was 41%. The survival was associated with response to therapy (p=0. 002); a similar result was obtained considering survival related to stable disease (SD). **Conclusions.** At the time of staging MDS patients, it's important to characterize the clinical condition of the patient, in order to keep the most appropriate therapeutic approach. AZA is an effective and safe drug, despite of the comorbidity score, resulting as a valuable option in frail patients. Time of response is longer than conventional therapies. In patients with aggressive disease it is not easy to reach an optimal number of cycles. Furthermore, our data suggest that achievement of SD is associated with survival advantage in MDS patients.

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BONE MARROW METASTASIS BY SOLID TUMORS: PREDICTION OF DIAGNOSIS AND PATIENTS OUTCOME

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Background. Bone marrow (BM) involvement is well-known complication of solid tumors witch generally affects treatment approach and patients outcome. **Aims.** In order to detect clinical and laboratory characteristics predicting spread to BM and survival of patients with metastatic malignancy, we analyzed and compared demographic data, localization of primary tumor and metastases, laboratory tests and BM aspirate and biopsy reports in patients with and without BM metastasis. **Methods.** We retrospectively evaluated data on 103 patients with metastatic solid tumors who underwent BM examination during the last decade. **Results.** Metastasis to BM was diagnosed in 72 cases, in 38 of them as a first evidence of malignancy. Pathologist was able to predict primary localization of the tumor in half of cases basing on BM biopsy only. Patients with and without BM involvement had comparable median age, gender distribution and pattern of the primary malignancy and metastatic spread. In univariate analysis, anemia (83% vs 61%; p=0. 015), thrombocytopenia (55% vs 16%; p<0. 001, with higher MPV 8. 73 vs 8. 17 fL p=0. 01, hypocellular/ dry tap marrow aspirate (58% vs 14%; p=0. 001) and leukoerythroblastic (LEB) blood smear (82% vs 0%; p<0. 0001) were found more commonly in patients with BM metastasis. In 92% of patients with LEB picture, the BM aspirate was hypocellular. At all, the marrow aspirate revealed cancer cells in 77% cases. The highest correlation rate of 93% between cytology and pathology reports was found in patients with prostate carcinoma. High (more than 2 upper limits, >2ULN) serum LDH and alkaline phosphatase levels were found more frequently in patients with BM metastasis (50% vs 16%; p=0. 002 and 35% vs 9%; p=0. 007, respectively). Multivariate analysis showed that thrombocytopenia <50x10^9/L (OR 14. 4; 95%CI, 1. 6-128; p=0. 02), hypocellular marrow aspirate (OR 7. 0; 95%CI, 1. 82-21. 2; p=0. 02) and alkaline phosphatase >2ULN levels (OR 6. 5; 95%CI, 1. 2-35. 3; p=0. 03) correlated with marrow involvement by biopsy. The diagnostic significance of the LEB picture was not calculated because of insufficient rate of records. Thrombocytopenia <50X10^9/L, LDH level >2ULN and primary presentation with BM metastasis vs BM spread occurring on follow-up were found to be main factors for poor outcome (median survival 0. 8 months (95% CI, 0. 1-1. 5) vs 4. 1m; (95%CI, 2. 0-6. 1); p<0. 001; 1. 8 months; (95%CI, 0. 5-3. 0) vs. 4. 7 months; (95%CI 3. 0-6. 3); p=0. 03 and 1. 8 months; (95% CI, 0. 3-3. 0 vs 4. 0 months (95%CI, 1. 3-6. 7); p=0. 03. **Conclusions.** Our results demonstrated that thrombocytopenia and high serum level of alkaline phosphatase correlate with BM metastasis in cancer patients. Although the diagnostic significance of the LEB picture was not calculated in the multivariate analysis, this finding correlated with hypoplastic aspirate that had been shown as a strong indication of BM involvement. Thrombocytopenia, high serum LDH and a finding of BM involvement at the time of malignancy primary diagnosis negatively affect overall survival

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APLASTIC ANEMIA IN PATIENTS OVER 60 YEARS OLD: EFFECTIVENESS AND SAFETY OF TREATMENT PROFILES

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Background. Aplastic anemia (AA) is a rare bone marrow failure syndrome characterized by peripheral pancytopenia and marrow hypoplasia. The incidence rate in patients over 60 years old is low. Due to the high morbidity and mortality related to bone marrow transplantation in older patients, immunosupresive therapy (IST) is the first line treatment. There is little evidence on the effectiveness and safety of IST in these patients. **Aims.** To describe the characteristics of patients over 60 years old with AA, and to analize the safety and effectiveness of treatment schemes, mortality rates and causes. **Methods.** We reviewed clinical histories of patients over 60 years old who suffer from AA at our hospital over the last four years. We collected data on age, sex, disease severity, treatment protocols and toxicities, type and duration of response to therapy, mortality and cause of death. Data source: clinical histories. Statistical desciption: non inferential because of the low level of patients. **Results.** Of 7 identified patients 1 was excluded due to incomplete follow-up. Of the 6 remaining patients all were female and they had a median age of 73 years (r, 63-87 years). 83% of them had a Charlson Index score of 2 or less (1 cardiovascular disease, 1 congestive heart failure, 1 peripheral vascular disease, 2 solid tumors and 2 cases of diabetes mellitus). 3 of the 6 patients had moderate AA, 1 severe AA and 2 very severe AA. 100% of patients received treatment with ciclosporin (CsA) in combination with other immunosuppresant drugs (in 3 cases associated with antithymocyte-globulin (ATG) and corticosteroids). After a median follow-up of 300 days (r, 90-1044 days), only 1 patient of the 6 responded to therapy, obtaining a partial response which lasted 953 days. Toxicities observed consisted of 3 cases of diabetes mellitus, 1 case of renal failure and 3 severe infections in three different patients. The only patient that responded to therapy developed a diffuse large B cell non-Hodgkin lymphoma 5 months after the diagnosis of AA, maintaining the acheived remission. At 15 months global survival was of 40% for non-responders, clearly inferior to the survival of the only responder. Causes of death were bacteremia, respiratory failure secondary to congestive heart failure and infection by Histoplasma capsulatum. **Conclusions.** AA is a disorder with a high mortality rate in over 60 year olds, but IST is generally well tolerated. While response rates are low, the

higher survival rates in responders clearly support the indication of IST, although the individualization of treatment decisions is fundamental due to the higher rate of co-morbidities in older patients.

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COMPLICATIONS OF 5-AZACYTIDINE: THREE CASES OF SEVERE COLITIS IN A SINGLE CENTER COHORT

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Background. The diagnosis of myelodysplastic syndrome (MDS) with high risk features according to the international prognostic scoring system (IPSS) results in a significant loss of lifetime in almost all patients, but allogenic stem-cell transplantation as the only curative option is not a valuable option for the majority of MDS patients due to age and co morbidity. 5-azacytidine (5-AZA) has shown a relevant benefit in terms of overall survival (OS) in patients with high-risk MDS in two randomized clinical phase III trials and based on these data about 549 patients 5-AZA was approved by the FDA in 2004 and by the EMA in 2008. The side effect profile of this drug seems favorable compared to conventional chemotherapy used for MDS or acute myeloid leukemia. However, rare side effects may have been overlooked in this cohort of patients. **Results.** Here we report a relevant incidence of hemorrhagic colitis in our single center cohort of 95 patients consecutively treated with subcutaneous 5-AZA between 2007 and 2011 in our tertiary cancer center within the Austrian Azacytidine Registry. Basic characteristics of the cohort have been presented in abstract form before (Pleyer et al.). 3 of 95 patients (3. 1%) developed severe colitis after a median of 1 month (range 1-2 months) of 5-AZA treatment. The median age was 81 years and 2 of the 3 patients were male. All had MDS with a high risk profile (2 cases of chronic myelomonocytic leukemia and 2 cases of refractory anemia with excessive blasts (RAEB)) and were treated with a median absolute dose of 140mg (100-150) for 7 days. Two of three patients had already known vascular complications before diagnosis of MDS (stroke and myocardial infarction). One patient was diagnosed with a severe hemorrhagic colitis at the end of the third cycle. The patient´s status deteriorated rapidly and after the diagnosis of colonic perforation colostomy had to be performed. Unfortunately, the patient developed a renal failure and died despite dialysis. Two other patients developed hemorrhagic colitis during or shortly after the first cycle of 5-azacytidine. After mesalazin treatment 5-AZA was continued in both cases after the resolution of the symptoms without further occurrence of colitis. **Conclusions.** To summarize we observed an incidence of 3 cases per 66. 8 person-years (4491 cases per 100. 000 person-years) in our cohort resulting in a much higher incidence compared with the incidence rates of colitis in the general population or in patients with older age and comorbidities (373 cases per 100. 000 person-years in patients older than 65 years with known COPD - Higgins et al. 2004). The reason for this relatively high incidence of colitis in our cohort is not clear. The reexposition in 2 patients without an adverse effect of 5-AZA makes a direct toxic effect on the mucosa or endothelial tissue very unlikely and we discuss whether the combination of anemia and constipation due to 5-HT(3) receptor antagonist and severe illness can explain the 3 cases of ischemic colitis. However, increased awareness for this complication in patients treated with 5-AZA should be warranted.

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SINGLE INSTITUTION EXPERIENCE OF 5-AZACITIDINE THERAPY IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA

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Background. In intermediate-2 and high risk myelodysplastic syndromes (MDS), chronic myelomonocitic leukemia (CMML) with 10-29% marrow blasts, and acute myeloid leukemia (20% to 30% marrow blasts) 5-azacitidine induces hematologic responses and prolongs overall survival. **Aims.** We retrospectively evaluated the efficacy and tolerability of azacitidine in patients with MDS, CMML and AML in our institution, outside a trial setting. **Methods.** from 2009 to 2012, 25 patients affected by int-2 and high risk MDS, CMML or AML with marrow blasts < 30%, who were not candidates to aggressive therapy, have been treated with azacitidine at a dosage of 75mg/m2/d subcutaneously 7 days (schedule 5-2-2) every 28 days. The WHO diagnoses were: 17 MDS with IPSS risk int-2 or high, 6 AML with blasts 20-30% and 2 CMML. The median age was 72 (range 37-81), male to female ratio was 0. 6 and the median number of cycles received was 7 (range 1-26). **Results.** We evaluated 17 (68%) patients, (13 MDS, 3 AML, 1 CMML) who had received 4 or more cycles (median number of cycles 8, range 4-26; median age 71, range 37-81, male to female ratio was 0. 8). The overall response rate (ORR) was 10/17 patients (59%). According to International Working Group (IWG) 2006 criteria, we detected complete remission (CR) in five patients (29%) after a median of 5 cycles (range 4-6), hematologic improvement with bone marrow complete remission (marrow CR) in two patients (12%) after 6 and 11 cycles of therapy, hematologic improvement (HI) in three patients (18%) after 5 cycles (range 4-6), stable disease (SD) in 4 patients (23%) and progressive disease (PD) in three patients (18%) after 5 cycles (range 4-7). Median duration of response was 12 months (range 6-26); median overall survival, for all patients treated, from the beginning of azacitidine was 14. 4 months (range 7-33). No differences in response rate could be appreciated according to age, bone marrow fibrosis, cytogenetic and transfusion requirements. Seven out of eight transfusion dependent patients (87%) acquired transfusion-independence. In the responder group (ten patients) we observed only two significant infectious complications (mucositis and sinusitis) and only one hospitalization (median observation time 10 months, range 5-33). Among the seven non-responding patients, four (57%) required several admissions to hospital because of infectious or hemorrhagic complications (median observation time 15 months, range 7-33). **Summary and Conclusions.** Besides a high rate of response (ORR 59%, CR 29%), azacitidine was well tolerated. In particular the low rate of serious adverse events and of hospital admissions despite severe cytopenias, greatly improved quality of life and reduced utilization of standard medical resources. Azacitidine confirmed to be an active therapy in patients not candidate to high intensity treatment for age and/or comorbidities.

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CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF MYELOFIBROSIS PATIENTS REFRACTORY TO CURRENT AVAILABLE THERAPIES IN MEXICO

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Background. Primary Myelofibrosis (PMF) post - polycythemia vera MF (PPV MF), and post - essential thrombocythemia MF (PET MF) are Myeloproliferative Neoplasms (MPN), characterized by bone marrow fibrosis. Since is considered as a rare disease, data from the North American Association of Central Cancer Registries and the Surveillance, Epidemiology, and End Results program have estimated the annual incidence of BCRABL1-negative diseases to be 2. 1 per 100,000, with an overall 3-year survival rate of 80%. Current incidence in Mexico is unknown. The use of aspirin, hydroxyurea, and phlebotomy for PV and ET, and the use of androgens, steroids, chemotherapy, and radiation therapy for PMF continue to be the mainstay of therapy. The only potentially curative therapy is allogeneic hematopoietic stem cell transplantation, but treatment-related mortality remains high. The discovery of the JAK2-V617F mutation in 2005 has triggered the era of targeted therapeutic approaches for MF including JAK inhibitors. This report will focus on the characteristics of 22 MPN patients refractory to current available therapies in Mexico. **Aims.** To describe the principal characteristics of MPN patients refractory to current available therapies and evaluate the usefulness of demographic Myelofibrosis Clinical Assessment in a multi-institutional cohort of Mexico. **Methods.** We collected data from 22 patients including age, gender, institution (public or private); liver and renal function, complete blood cell count (CBC); subtype of disease; risk group, ECOG performance status, spleen size, and bone marrow fibrosis grade. **Results.** The median age was 63. 6 years; 47. 4% were female, 58% of patients proceeded from Private Health Institutions (PHI). Liver and renal function were adequate in 100% of patients, defined as direct bilirubin ≤ 2. 0 x ULN, and serum creatinine ≤ 2 x ULN; CBC analysis presented a peripheral blood blast count < 10%. 42. 1% of patients had PMF; 21% had PPV-MF; and 36% had PET-MF. 11% had Low risk; 53% intermediate-1 risk; 26% intermedi-

ate-2 risk; and 10% high risk. ECOG 0 was present in 21% of patients; ECOG 1, 63. 16%; ECOG 2, 10. 53%; and ECOG 3 in 5. 3%. Splenomegaly was found in 100% of cases; with 63% of splenomegaly less than 10cm; 26. 36% between 10 and 20cm; and 10. 53% more than 20cm. Spleen medium size was 10. 3 cm; BLCM. Bone marrow biopsy showed: No Fibrosis, 5%; Grade I, 42%; Grade II, 21%; Grade III. 32%. (Figure 1). **Conclusions.** Characteristics of MF patients of this multi-institutional cohort in Mexico, remark the disease's severity. The median age of patients was according with the literature reported (66 y. o.). In this cohort, most patients were from Private Health Institutions. And PMF was more frequent than PPV-MF and PET-MF.

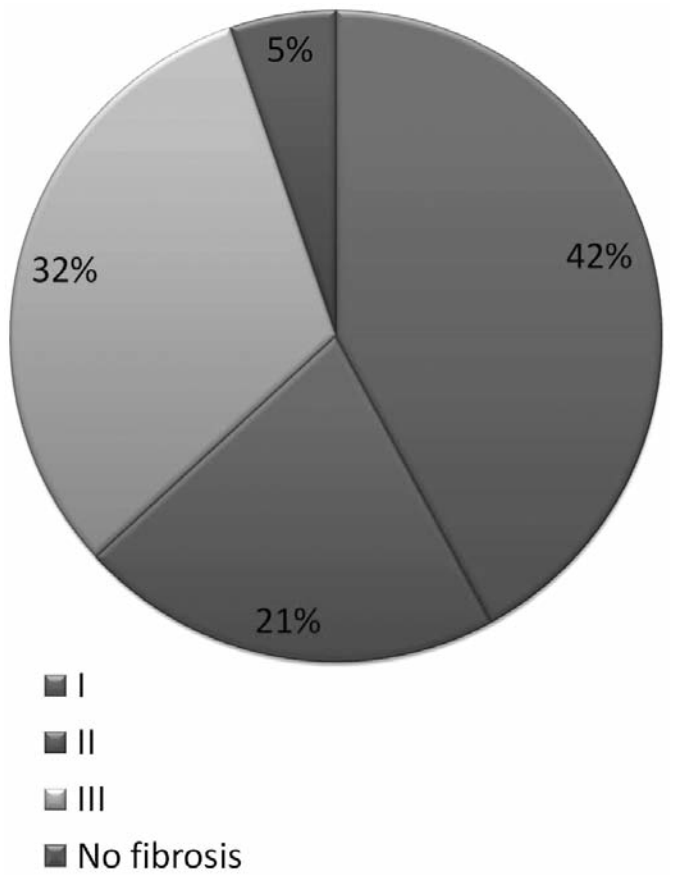

Figure 1. Grade of bone marrow fibrosis in patients with myelofibrosis in Mexico.

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TREATMENT OF CHRONIC MYELOMONOCYTIC LEUKEMIA WITH 5-AZACITIDINE: A CASE SERIES

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Background. Chronic myelomonocytic leukemia (CMML) is a clinically heterogeneous clonal disease, recently classified as a mixed myelodysplastic/myeloproliferative disorder. Life expectancy is highly variable and there is currently no standard treatment. Stem cell transplantation remains the only potentially curative therapeutic strategy but only in highly selected patients. The previously documented hypermethylation of some genes in CMML indicates that hypomethylating agents could be an adequate targeted therapy in these patients and the efficacy of these agents is currently being tested in clinical trials. **Aim.** To evaluate the efficacy and toxicity profile of 5-azacitidine (AZA) in CMML patients. **Methods.** A retrospective analysis of patients diagnosed with CMML and treated with AZA in our institution between 2005 and 2010 was conducted. Diagnosis was established according to the World Health Organization 2008 criteria. AZA was administered subcutaneously, either as a seven-day regimen (75mg/m^2/day) or a five-day regimen (100mg/m^2/day), every 4 weeks. Patients received transfusions, antibiotics, and other supportive therapies, at the physician's discretion. Marrow response was assessed following the 6th treatment cycle and subsequent cycles were administered should there have been absence of disease progression. Responses were assessed according to the modified IWG criteria. **Results.** A total of eight patients were identified, with a median age at diagnosis of 66 years. Five were classified as non-proliferative CMML and the remaining as proliferative CMML. Seven cases fulfilled criteria for CMML-1 and one for CMML-2. Three patients had secondary CMML. All patients were transfusion dependent at diagnosis or soon after starting cytoreduction. Prior to AZA, five patients received cytoreductive therapy, one patient received erythroid stimulating agents, one thalidomide and other interferon, without any reduction in transfusion requirements. The overall response rate to AZA was 62. 5% (5 of 8), with 37. 5% complete responses (CR) and 37. 5% of cases with transfusion independence. Of note, a significant response rate was obtained in patients with proliferative CMML, with good control of leukocytosis and transfusion independence documented in 2/3 of these patients. With a median follow-up of 12 months, the median survival (from start of therapy) was 10 months and three patients are still being treated with AZA. Four patients have died, three from disease progression. Treatment was generally well-tolerated with no reported grade 3 or 4 toxicity except for one case of febrile neutropenia requiring hospital admission. **Conclusions.** In our series, AZA treatment was associated with an overall response rate of 62. 5%. Patients who achieved a CR had longer response durations. Interestingly, a significant response rate was also obtained in patients with proliferative CMML, encouraging the use of AZA in all forms of this disease. As the molecular pathogenesis of CMML is further elucidated, the number of available pharmacological treatment strategies will increase, leading to improved patient quality of life and outcomes.

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AUTO-IMMUNE HEMOLYTIC ANAEMIA AS INITIAL PRESENTATION OF MYELODYSPLASTIC SYNDROMES: REPORT OF THREE CASES

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Background. auto-immune hemolytic anaemia (AIHA) represent a common manifestation of lymphoid disorders. **Aims.** we report three cases of AIHA associated with myelodysplastic syndromes (MDS). Two of them had favourable evolution either on specific MDS therapy or on corticosteroid therapy. **Case Report.** First case: 70 yrs old man developed one year after the diagnosis of MDS an acute anaemia with hemolytic features, biological tests showed red cell with auto-antibody. Outcome was good after 6 months period of corticosteroid therapy and slowly decrease of the dose. One year after the begining on therapy, no marker of relapse wes seen. Second case: 80 years old women was sent for severe anaemia due to 5q minus syndrome. Some hemolytic parameters revealed AIHA, corticosteroid was ineffective but lenalidomide at 5mg per day was effective in 15 days. One years after treatment discontinuation (polyglobulia) she remains in complete remission. Third case: 73 years old man with symptomatic anaemia. A the same time the diagnosis of RAEB-t (AML with 23% blasts) was performed. Irregular auto and alloantibodies were present. No transfusion was possible (severe reactions), no treatment gives results for the dinner. The patient died 15 years later with 2g/dl of hemoglobin level. **Conclusions.** evolutive profiles of AIHA in MDS differ from other situation. Corticotherpy are often uneffective (66%of cases).

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5-AZACITIDINE TREATMENT RESTORES RESPONSE TO EPOETIN THERAPY IN HIGH RISK MYELODYSPLASTIC (MDS) PATIENTS

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5-Azacitidine(5-Aza) is an extremely valid therapeutic option in int-2/high risk MDS patients who can not undergo a bone marrow transplant procedure and is able to induce hematological responses and reduce the progression to acute myeloid leukemia in most of patients. Epoetin(Epo)therapy, differently by low risk MDS patients who obtain a high rate of erythroid responses, has no role in the setting of higher risk patients. Cause of the 5-Aza characteristics the response to the drug is not rapid but it generally takes at list three cycles to become evident with a progressive hematological and QoL improvement. For these reasons we have thought that the administration of Epo in int-2/high risk MDS patients who are on 5-Aza treatment and who begin to show some sort of erythropoietic response(expression of the MDS clone proliferative advantage reduction and the normal hematopoiesis expansion)could help to accelerate the hemoglobin rise, with a more rapid improvement in patients QoL. Three int-

2,three high-risk MDS patients and one AML patient have been treated with 5-Aza at our institution; some of them had a history of Epo therapy with no response. All patients were transfusion-dependent when they started 5-Aza. We decided to begin Epo therapy as soon as the patients showed some sort of initial erythroid response(Hb ≥ 8 g/dl for three consecutive determinations and an initial reduction in transfusional support need). All but one patient received α-Epo at a dose of 40. 000 IU/wk. All patients have shown a response to Epo (only one patient had to increase the dose to a twice weekly schedule)with a rapid improvement in QoL; in two of them the response has been extremely rapid and so impressive that we had to stop the drug. What is surprising is that these two patients had a very poor prognosis:- A 72 years old man with a high-risk MDS(complex cariotype, severe citopenia,high transfusional need:6 red blood cell unit/month)- A 60 years old female with a high-risk MDS secondary to high dose chemotherapy and bone marrow transplantation for NHL(-7 cariotype, severe citopenia and transfusional need:3 red blood cell unit/month)Cause of the low number of patients and the fact that it has been just a clinical study, it is not possible to draw any reasonable Conclusions. But as a regard to the results that we obtained it is our department policy to begin Epo therapy in the setting of int-2/high-risk MDS patients who are on 5-Aza and begin to show some sort of erythroid response

1480

IDENTIFICATION OF UNBALANCED GENOME COPY NUMBER ABNORMALITIES IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Single nucleotide polymorphism genotyping microarray (SNP array) provides detailed information on chromosomal copy number imbalances. With such information, in addition to that from conventional chromosomal analysis, knowledge about the molecular pathogenesis of hematological malignancies including multiple myeloma (MM) has grown substantially.

Table 1. Frequencies and prognostic impact of chromosomal copy number abnormalities.

Copy number abnormality	Cases, n (%)	Prognosis	*p* value
1q+	20 (51.2)	-	0.082
3q+	12 (30.7)	-	0.893
5q+	10 (25.6)	-	0.716
6p+	8 (20.5)	-	0.538
7q+	12 (30.7)	-	0.618
9q+	16 (41.0)	-	0.223
11q+	15 (38.4)	-	0.365
15q+	15 (38.4)	-	0.058
ch 18+	5 (12.8)	-	0.550
19q+	17 (43.5)	-	0.065
del(8p)	11 (30.7)	-	0.155
del(13q)	15 (38.4)	poor	0.049*
del(16q)	6 (15.3)	-	0.190
del(17p)	5 (12.8)	-	0.118
del(22q)	7 (17.9)	-	0.217
All UPDs	17 (43.5)	-	0.193
NHD vs HD	16 (41.0)	poor	0.039*
NHD with 1q+ vs NHD without 1q+	8 (50.0)	poor	0.013*
HD with 1q+ vs HD without 1q+	12 (52.1)	-	0.965
NHD with del(13q) vs NHD without del(13q)	9 (56.2)	-	0.099
HD with del(13q) vs HD without del(13q)	6 (26.0)	-	0.542
deletion and UPD of *SEZ6L* (22q12.1)	11 (30.7)	-	0254.
HD with deletion or UPD of *SEZ6L* [(a)]	5 (27.7)	poor	0.040*
NHD with deletion or UPD of *SEZ6L* [(b)]	6 (37.5)	-	0.497

*indicates significant *p* value ($p < 0.05$).

+ indicates gain of chromosome.

(a) Compared with HD without deletion or UPD of *SEZ6L*.

(b) Compared with NHD without deletion or UPD of *SEZ6L*.

Abbreviations: ch, chromosome; del, deletion of chromosome; UPD, uniparental disomy NHD, nonhyperdiploid; HD, hyperdiploid; vs, versus *SEZ6L*, seizure related 6 homolog (mouse)-like

Aims. To investigate detailed genetic abnormalities which are related to pathogenesis of MM, we performed higher resolution SNP array which can provide more information on chromosomal copy number imbalances with higher quality. **Methods.** Genomic DNA, obtained from 39 bone marrow samples of MM patients and 11 cell lines, were analyzed by using 250K GeneChip SNP genotyping microarrays. The array data were analyzed with the copy number analyzer for GeneChips (CNAG) software for allele-specific copy number analysis (http://www. genome. umin. jp). The patients' clinical information were retrospectively obtained from the medical records. Kaplan-Meier survival curves were calculated using the SPSS software. **Results.** We identified accumulation of previously unidentified deletions and uniparental disomies in 22q12. 1. Among hyperdiploid MM, chromosomal imbalance of this locus provided poor prognosis ($p = 0.040$). By sequencing, a mutation was found in the seizure related 6 homolog (mouse)-like (*SEZ6L*) gene located at ch. 22q12. 1 in an MM cell line, NOP1. We further found isolated deletions in 17 genes, five of which are known tumor suppressor genes. Among these, deletion of protein tyrosine phosphatase, receptor type D (*PTPRD*) was found in 3 samples, including 2 from patients. Consistent with previous reports, non-hyperdiploid MM, deletion of 13q, and among non-hyperdiploid MM, gain of chromosome 1q were predictive of poor prognosis ($p = 0.039$, $p = 0.049$, and $p = 0.013$, respectively). However, our analysis revealed that unless accompanied by gain of chromosome 1q, the prognosis of non-hyperdiploid MM is comparable to that of hyperdiploid MM. **Conclusions.** We could find some candidate chromosomal copy number imbalances which might be related to pathogenesis and prognosis of MM. SNP array analyses provide significant information toward understanding the pathogenesis and prognosis of MM.

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IMMUNOPARESIS CORRELATES WITH MGUS RISK - APPLICATION OF POLYCLONAL IMMUNOGLOBULIN AND HEAVY/LIGHT CHAIN LEVELS ASSESSMENT

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Backround. Monoclonal gammopathy of undetermined significance (MGUS) indicates an asymptomatic and potentially malignant state characterised by benign clonal proliferation of plasma cells secerning monoclonal immunoglobulin (MIG, M-protein) in the absence of malignant lymphocyte proliferation. It has been observed that in some patients, MGUS transforms into one of the malignant forms of monoclonal gammopathy. The well-known factors determining the risk degree of progression include in particular the quantity, M-protein type and the ratio of immunoglobulin free light chains (FLC) allowing the efficient stratification of MGUS patients. In addition, several authors consider the present suppression of polyclonal immunoglobulin levels to be a potential factor of progression. **Aims.** The study aimed at conducting a analysis of polyclonal immunoglobulin and heavy/light chain pair (HLC) levels in a group of MGUS patients. The polyclonal immunoglobulin levels and the levels of HLC alternative pairs were to be compared with a view to verify the degree of immune paresis depending on the MGUS risk degree. **Methods.** The analysed set comprised 130 serum samples of MGUS patients (102 IgG, 28 IgA) who were stratified into 4 risk groups (low, low-intermediate, high-intermediate, and high risk of transformation) according to the levels, M-protein type and FLC index values. FLC levels, polyclonal immunoglobulin levels, and HLC levels were determined by means of the SPA Plus turbidimeter platform. In the statistical analysis, Mann-Whitney U Test with Bonferroni correction was applied. **Results.** Comparison of the suppression degree of polyclonal immunoglobulin levels in individual risk classes in the IgG MGUS group brought significantly higher levels of IgA immunoglobulin in patients with low or low-intermediate risk as opposed to the group with high-intermediate risk (p = 0. 0001 and p = 0. 047 respectively). When analysing the MGUS group with IgA isotype, notably higher levels of IgM immunoglobulin were evident in the group with low-intermediate risk than in patients with high-intermediate or high risk (p = 0. 035 and p =

0. 017 respectively). In addition, the levels of alternative isotype pairs were compared among the individual risk groups of the IgG MGUS group. Patients with dominant IgGκ secretion showed significantly higher levels of the IgGλ alternative pair in the group of low and low-intermediate risk than in the group of high-intermediate risk (p = 0. 003 and p = 0. 006 respectively). In case of dominant IgGλ secretion, considerably higher IgGκ levels were detected in patients with low or low-intermediate risk than in the group of high-intermediate risk (p = 0. 0006 and p = 0. 009 respectively). **Conclusions.** The discovered connection between the degree of immune suppression depending on the MGUS risk level and especially the clearly visible benefit of determining the alternative HLC pairs contributes with another aspect to understanding the links between the biology, behaviour, and the potential malignant evolution of MGUS with the advantage of obtaining a well-measurable parameter. Supported by NT 12451/5, NS 10387 and The Binding Site, Czech Republic.

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GENE EXPRESSIONS IN WHOLE BONE MARROW SAMPLES AND ASSOCIATION WITH OSTEOLYTIC BONE DISEASE IN MULTIPLE MYELOMA PATIENTS

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Background. The multiple myeloma (MM) plasma cell (PC) is dependent on the BM microenvironment. Most gene expression studies have been performed upon BM aspirates, which differ in cellular composition, or MACS isolated PCs. We used a new strategy to analyze gene expressions in snap-frozen BM biopsies, hereby coming close to what is "actually going on in the BM of MM patients". We analyzed the gene expression of known bone-regulating cytokines that predominantly are synthesized by stromal cells and that may be involved in the pathophysiology of myeloma osteolytic bone disease (OBD). **Aims.** To investigate the quantitative expression levels of bone-regulating cytokines: SDF1, GDF15, IL6 and IL6R, IL11, MIP1α and FGF2 in whole BM biopsies.

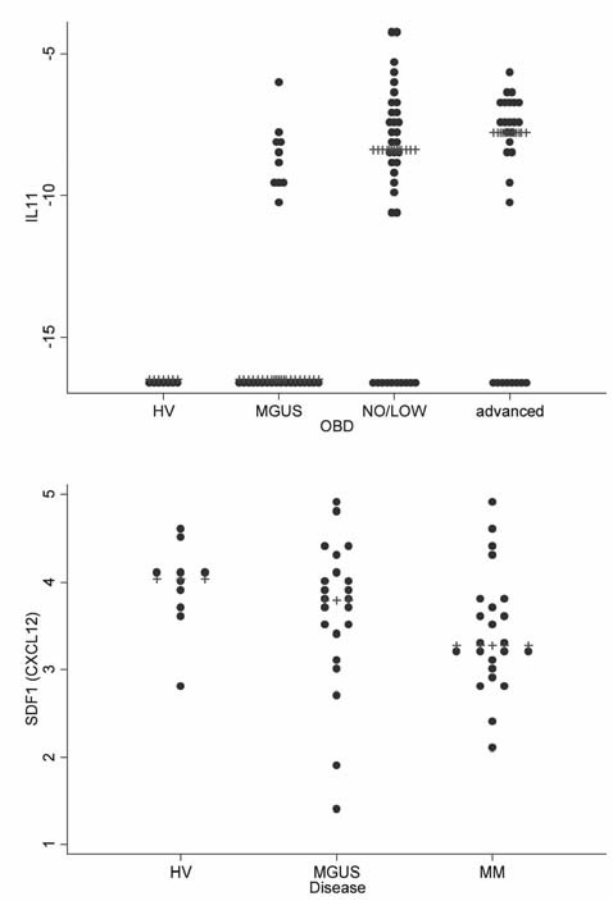

Fig 1. Delta Ct of significant genes.

Methods. During the diagnostic procedure we obtained an extra BM core biopsy which was snap-frozen. Biopsies where cut, homogenized and RNA was purified using the MagNa Pure Robot (Roche). cDNA was loaded for QPCR on 384-wells micro-fluidic cards (Applied Biosystems). Using 3 internal reference genes (ABL, GAPDH and GUS) the relative quantitative gene expression was calculated. OBD was evaluated using standard radiographs. All patients were untreated and did not receive bone-remodeling medicine. We examined 10 HV, 41 MGUS, and 71 untreated MM patients. Based on radiographic findings OBD was divided into NO/LOW and advanced, i. e. OBD in ≥2 regions. **Results.** The gene expression of IL-11 was significantly (p<0. 05) associated with OBD with increasing gene-expression with increasing OBD. FGF2, IL-6, GDF15, MIP1α and IL6R were not associated to OBD. CXCL12 associated significantly to disease state (HV vs MGUS vs MM). FGF2, IL-6, GDF15, MIP1α, and IL6R did not associate to disease stage (HV, MGUS or MM). **Discussion and Conclusions.** In our gene expression study reflecting the *in vivo* situation in MM, IL-11 was significantly associated to OBD. The use of whole snap-frozen BM core biopsies is a new method to evaluate gene expression in MM offering information on what is going on in the microenvironment. Furthermore, it is possible to investigate patients independent of degree of MM PC infiltration.

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IGA HEAVY/LIGHT CHAIN ANALYSIS - A NEW MARKER FOR THE DIAGNOSIS AND MONITORING OF MYELOMA PATIENTS

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Multiple myeloma diagnosis and follow up are based on bone marrow plasma cell enumeration and monoclonal protein measurement. The estimation of monoclonal immunoglobulin production requires serum protein electrophoresis (sPE), serum immunofixation (sIF) and serum free light chain assay. A new immunoassay has been developed, based on the recognition of epitopes spanning the junction of the immunoglobulin's heavy and light chains, therefore allowing the measurement of IgA kappa (IgAk) and IgA lambda (IgAλ) heavy/light chain pairs. The aim of this study was to determine the clinical utility of this new assay for the diagnosis and follow up of myeloma patients. Ten non myeloma patients and 23 patients with IgA monoclonal gammopathies were enrolled. Total IgA, IgAk, IgAλ assays, sPE and sIF were performed at diagnosis and during follow-up. Post-treatment bone marrow residual disease was assessed by flow cytometry. At diagnosis, all myeloma patients had an abnormal IgAk/IgAλ ratio allowing a good discrimination between myeloma and non myeloma patients. During the monitoring of monoclonal immunoglobulin, we compared the concentration of IgAk or IgAλ to monoclonal spike estimation given by sPE when migrating in the γ region. The values of IgAk or IgAλ monoclonal isotypes gave an evolution profile that correlated well (R^2=0. 956) with the densitometric estimation of the monoclonal spike. For 2 patients who relapsed, the IgAk/IgAλ ratio became abnormal before sPE and sIF suggesting that this ratio could be an early marker for the disease relapse. For 4 patients under remission, the IgAk/IgAλ ratio was in accordance with the negative flow cytometry bone marrow residual disease evaluation, while the sIF was still slightly positive in 2 patients and became negative at the same time for the third patient or a month later for the fourth one. Concerning patients with disease progression (3 myeloma patients and 1 smoldering myeloma patient) we observed a progressive increase of the monoclonal isotype associated with the concomitant decrease of the non monoclonal isotype. The measurement of IgAk and IgAλ concentrations and the ratio evaluation gave clear information on the disease evolution. In conclusion, IgA heavy/light chain assay is an interesting tool that brings more insights in the myeloma biology. It provides automated, reproductible measurements of heavy/light chains of immunoglobulins. The values of IgAk or IgAλ can be used for monitoring myeloma. This will be even more useful when the monoclonal IgA comigrates on sPE with normal proteins in β region making impossible a reliable densitometric estimation. Moreover, the assay provides supplementary information on the non monoclonal isotype suppression or recovery. The very sensitive IgAk/IgAλ ratio provides an early appreciation of the response or relapse status.

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HEVYLITE AS A BIOMARKER FOR MONITORING PATIENTS WITH IGM MONOCLONAL GAMMOPATHY AND ANTI-MAG NEUROPATHY

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Background. Patients with neuropathy associated with anti-myelin-associated glycoprotein (MAG) antibodies and monoclonal IgM gammopathy constitute a distinctive clinical syndrome. Measurement of serum M-spike is used to monitor these patients; however, its resolution on serum protein electrophoresis (SPE) is sometimes impossible or inaccurate. The same applies to total IgM quantification by nephelometry (IgMneph). Recently, a new immunoassay, Hevylite® (Binding Site, Birmingham, UK), was developed to quantify separately IgMκ and IgMλ. The IgMκ/IgMλ ratio (HLC ratio) might provide true quantitative measurement of the IgM M-spike evaluating the involved monoclonal IgM compared to uninvolved polyclonal IgM. We sought to determine whether Hevylite™ assay can be used as a reliable marker for patients with anti-MAG neuropathy at diagnosis of IgM monoclonal gammopathy and to evaluate response to therapy as compared to the other tests currently available. **Methods.** The study was conducted in a series of 44 patients with anti-MAG neuropathy (30 monoclonal gammopathy of undermined significance (MGUS) and 14 Waldenström's Macroglobulinemia (WD)). All serum samples were collected prior and after chemotherapy (Chlorambucil or Rituximab) and were kept frozen at -80°C until subsequent use. Clinical response was defined as neuropathy stabilization or improvement after chemotherapy. M-protein was detected and quantified by SPE, then identified by Immunofixation electrophoresis (IFE) (Sebia, Evry, France). IgM anti-MAG antibodies were evaluated by ELISA (Bühlmann Laboratories, Basel, Switzerland). Hevylite™ measurements were performed by tubidimetry on SPA_{PLUS} analyser according manufacturer recommendations and compared with the normal range determined from normal sera (blood donors, n=147). Median (95th percentiles) were: IgMκ 0. 71g/L (0. 19-1. 63), IgMλ 0. 39g/L (0. 12-1. 01), HLC ratio 1. 81 (1. 18-2. 74). For ease of comparison, we mainly studied HLC ratio. **Results.** Before treatment, all 44 patients had monoclonal IgM detected by SPE and IFE, with only 24 (55%) having an IgM M-spike quantifiable on SPE. Thirty-nine patients (89%) had a monoclonal IgMκ and 5 a monoclonal IgMλ. All patients had anti-MAG antibody level higher than 1000 BTU (Bühlmann titer units) and abnormal HLC ratio using Hevylite™. For patients with IgMκ, a ROC curve showed that HLC ratio had 89% specificity, 42% sensitivity and AUC of 0. 72 to distinguish patients with WD from those with MGUS, for a cut-off ratio of 60. Thirty-eight patients (86%) responded clinically. In before/after treatment comparisons, neither HLC ratio (p=0. 83) nor M-spike on SPE (p=0. 74) were associated with disease course after chemotherapy, whereas we observed a significant decrease of anti-MAG activity for patients with clinical response (p=0. 04). **Conclusions.** We assessed the diagnostic and prognostic value of Hevylite™ assay in anti-MAG neuropathy associated with IgM MGUS or WD. Current laboratory assays have some limitations: IFE cannot quantify IgM; SPE and IgMneph are not sensitive enough to evaluate accurately involved IgM; anti-MAG activity is frequently above the limit of quantification. HLC ratio has the advantage of providing numerical results for all patients, especially for monitoring patients with MGUS. Hevylite™ needs further validation and might become the reference technique to monitor the IgM M-protein associated with anti-MAG activity.

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ANALYSIS OF CHROMOSOMAL ABNORMALITIES ASSOCIATED WITH HIGHER RISK OF MALIGNANT TRANSFORMATION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background. Monoclonal gammopathy of undetermined significance (MGUS) is a precancerosis permanently associated with higher risk of transformation into malignant disease. Similarly to multiple myeloma patients, specific chromosomal aberrations in bone marrow plasma cells (PCs) in MGUS persons were reported, especially del(13)(q14), del(17)(p13), *IGH* rearrangement, 1q21 gain and hyperdiploidy (Lopéz-Corral, L. *et al.,* Clin. Cancer Res. 2011). Some of them are considered to be prognostic factors determining potential risk of MGUS transformation to malignant condition. **Aims.** 1) Examination of specific chromosomal abnormalities in the MGUS persons by I-FISH. 2) Assessment of impact of phenotypically abnormal PCs (aPCs; $CD138^+CD19^-CD56^{+/-}$) separation on the frequency of monitored chromosomal abnormalities. 3) Evaluation of potential prognostic value of the observed chromosomal abnormalities. **Methods.** We analyzed 60 MGUS persons: 36M/24F; age median 64 years; follow-up median 26 months. By interphase fluorescence *in situ* hybridization (I-FISH) we examined following chromosomal abnormalities: del(13)(q14), del(17)(p13), *IGH* rearrangement, t(11;14), t(4;14), t(14;16), 1q21 gain and hyperdiploidy (+5, +9 and +15). The I-FISH was performed on PCs i. identified by fluorescence imunophenotyping (cIg-FISH technique) in 3% (2/60) cases; ii. separated by magnetic activated cell sorting (MACS) or fluorescence activated cell sorting (FACS) using $CD138^+$ marker in 63% (38/60) cases; and iii. separated by FACS using $CD138^+19^-56^{+/-}$ markers in 33% (20/60) cases. **Results.** In the cohort of 60 MGUS persons, we found del(13)(q14) in 21% (11/52) cases, del(17)(p13) in 2% (1/52) cases, *IGH* rearrangement in 71% (31/44) cases, t(11;14) in 50% (2/4) cases, t(4;14) in 10% (4/39) cases, t(14;16) in 6% (1/16) cases, 1q21 gain in 13% (6/48) cases and hyperdiploidy in 15% (7/47) cases. We observed that del(13)(q14) occurred significantly more frequently in persons with hyperdiploidy than with non-hyperdiploidy (67% vs. 17%, p<0,05), and in persons with 1q21 gain than without 1q21 gain (67% vs. 16%.; p<0,05). Comparison of MGUS subgroups with different PCs phenotype enabled to us to show that I-FISH analysis in aPCs allowed to significantly higher frequency of t(4;14) than I-FISH analysis in PCs with CD138+ phenotype (25% vs. 0%; p<0,01). Using stratification model for predicting the risk of MGUS transformation incorporating 3 risk factors (serum monoclonal immunoglobulin ≥15g/l, non-IgG subtype and abnormal FLC ratio) (Rajkumar, S. *et al.,* Blood 2005). MGUS persons were divided into three risk groups: low risk was in 27% (9/33) cases, low-intermediate risk was in 39% (13/33) cases and high-intermediate risk was in 33% (11/33) cases. High risk was not detected in any case. Analysis of frequency of monitored chromosomal abnormalities demonstrated that del(13)(q14) and two or more structural chromosomal abnormalities occurred significantly higher in low-intermediate risk group (45% and 46%) and high-intermediate risk group (33% and 18%) than in low risk group (both 0%; p<0,05). **Summary.** In this study, we confirm that all monitored chromosomal abnormalities, which usually occur in multiple myeloma, are present in MGUS. We also demonstrated that del(13)(q14) or presence of two or more structural chromosome abnormalities may indicate the higher risk of MGUS transformation to malignant condition. **Support.** The study was supported by grants MSM0021622434, GAP304/10/1395, NT11154, NT12425, NT12130.

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BORTEZOMIB DOWNREGULATES THE EXPRESSION AND PRODUCTION OF STROMAL CELL-DERIVED FACTOR-1 IN BONE MARROW STROMAL CELLS

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Background. Stromal cell-derived factor-1 (SDF-1) is a chemokine that is expressed constitutively and produced in bone marrow stromal cells (BMSCs). Myeloma cells express CXCR4 and respond to SDF-1, resulting in the trafficking and localisation of these cells in the bone marrow (BM). SDF-1 alone has minimal or negligible effects on the survival and growth of myeloma cells *in vitro*. However, many reports are consistent with the SDF-1/CXCR4 axis being involved in the progression of myeloma and suggest that modulation of the SDF-1/CXCR4 axis could influence the biology of myeloma cells and the disease course. Bortezomib induces apoptosis and strongly inhibits the proliferation of myeloma cells, although the effects of bortezomib on BMSCs are not clear. We hypothesised that bortezomib affects the expression or production of SDF-1 in BMSCs. **Aims.** To define the effects of bortezomib on the expression and production of SDF-1 in BMSCs derived from both healthy individuals and patients with multiple myeloma. **Methods.** We investigated bortezomib-induced alterations in the expression and production of SDF-1 in the bone marrow stromal cell line MS-5 and in BMSCs obtained from three healthy individuals and three patients with multiple myeloma. The expression of SDF-1, CXCR4, and CXCR7 mRNA was analyzed using reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time quantitative (RQ)-PCR. The production of SDF-1 was measured using Western blotting and an enzyme-linked immunosorbent assay (ELISA). **Results.** In a 3-day incubation of MS-5 cells, low-concentration (5 nM) bortezomib did not affect cell proliferation, whereas ≥50 nM bortezomib abolished cell proliferation. By contrast, bortezomib concentrations ≤500 nM did not affect apoptosis of the MS-5 cells over the 3-day period. Similar results were obtained with BMSCs from both healthy individuals and patients with myeloma. RQ-PCR revealed that bortezomib induced the downregulation of SDF-1 mRNA in a dose-dependent manner in MS-5 cells. Treating the cells with bortezomib at concentrations of 5, 50, and 500 nM for 24 h reduced the SDF-1 mRNA to 50, 20, and <10% of the control, respectively. Similar results were obtained with BMSCs from healthy individuals and patients with myeloma. Bortezomib induced the downregulation of SDF-1 production in a

dose-dependent manner in both MS-5 and BMSCs, as analyzed by Western blotting and confirmed by measuring SDF-1 concentrations in the 3-day culture supernatants of the cells. In line with these results, the supernatants from bortezomib-treated MS-5 cells induced MO7e cell chemotaxis to a much lower extent compared with non-treated cells. MS-5 cell monolayers were treated with bortezomib for 24 h, washed, and then overlaid with RPMI8226 cells. The migration of RPMI8226 cells under the monolayer was checked 24 h later, and the number of cobblestone areas was checked 5 days later. Both were reduced significantly in the bortezomib-treated monolayers compared with the non-treated monolayers, indicating that bortezomib-induced downregulation of SDF-1 was functionally active *in vitro*. **Conclusions.** Bortezomib affects not only myeloma cells but also BMSCs. Most notably, it downregulates the expression and production of SDF-1 in the cells irrespective of the presence of myeloma.

1487

HEAVY/LIGHT CHAIN (HLC) NEPHOLOMETERIC ASSAYS EFFECTIVELY MONITORS OLIGOSECRETORY MULTIPLE MYELOMA PATIENTS WHO CANNOT BE MEASURED BY OTHER SEROLOGICAL MARKERS

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Background. Effective monitoring of multiple myeloma (MM) patients enables physicians to accurately assess patient's responses to therapy and make timely decisions regarding treatment changes. Approximately 1% of MM patients are oligosecretory, defined as less than 10g/L monoclonal immunoglobulin. Monitoring these patients using standard electrophoretic techniques represents a significant challenge. The introduction of the polyclonal serum free light chain (FLC) test (Freelite®) has improved the ability to monitor these patients and international guidelines now recommend their use when >100mg/L FLC can be measured at presentation. However, in a small subset of patient's FLC testing is of limited utility. Here we assess the utility of novel IgAk / IgAl nephelometic immunoassays and comment on their utility in oligosecretory patients. **Aims.** To assess the utility of HLC IgA measurements to monitor patients with IgA oligosecretory MM. **Methods.** HLC and FLC assays were performed retrospectively on stored sera from 11 oligo-MM patients (5 IgAk and 6 IgAl). Patients were followed from start of therapy and monitored in regular intervals during the course of their disease. **Results.** 5/11 IgA oligo-MM patients had iFLC >100mg/L at presentation (median 298mg/L, range 125-6700mg/L); each patient achieved a partial response (PR; >50% reduction FLC concentrations). 6/11 IgA patients had iFLC<100mg/L at presentation (median 32mg/L, range 16-57mg/L); all 6 of these patients had an abnormal HLC ratio at presentation. Overall, changes in HLC ratios were in accordance with the clinical assessment during patient monitoring. 5/6 patients responded to therapy; at maximum response 3 patients achieved a PR, 1 patient achieved a very good partial response (VGPR) and 1 patient achieved a near complete response (nCR). In each case the HLC IgA ratio reduced but remained abnormal. The remaining patients failed to respond to therapy, and maintained a stable, abnormal HLC ratio throughout monitoring. In 3/6 patients (2 IgAk / 1 IgAl), increasingly abnormal HLC ratios identified disease progression before either an increase in monoclonal proteins was identifiable by SPE (median: 195 days, range 104-445 days). One IgAk patient achieved a VGPR after 284 days; the response was maintained for 168 days before a relapse was identified by a 101% increase in monoclonal immunoglobulin. Increases in the HLC ratio indicated a relapse after 340 days, 112 days earlier than the traditional assessment. The patient responded to second line therapy and achieved a second VGPR after 601 days. Again increasingly abnormal HLC ratio identified a second relapse 104 days earlier than traditional assessment confirmed progression. In a second IgAk patient achieved a PR after 1941 days and increasingly abnormal HLC ratio identified relapse after 2341 days, 445 days before clinical assessments. Finally, in 1 IgAl patient who initial failed to respond to therapy, achieved a PR after 510 days. An increase in the patient's HLC ratio after 560 days indicated disease progression, 120 days before clinical assessments. **Conclusions.** HLC analysis allows the sensitive detection of monoclonal protein in oligosecretory MM patients. This sensitivity is a reflection of both the tumor production and isotype matched suppression and may aid in oligosecretory patient monitoring.

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ANALYSIS OF SERUM LEVELS OF THYMIDINE KINASE IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA COMPARISON WITH IMAGING Methods 99MTC-MIBI SCINTIGRAPHY AND 18F-FDG PET/CT

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Background. Proliferation is a independent prognostic factor and target for risk adapted treatment in multiple myeloma. Increased activity of thymidine kinase in serum is associated with higher proliferation rate in hematological malignancies. The imaging methods as 99mTc-MIBI scintigraphy or 18F-FDG PET/CT can imagine active myeloma lesions and their positivity is negative prognostic factor. **Materials and Methods**The analyzed group of 51 patients consisted of 14 (27,5%) individuals with monoclonal gammopathy of undetermined significance (MGUS) and 37 (72,5%) patients with multiple myeloma (MM). There were 25 female (49%) a 26 male (51%) in the group. The average age in the group was 63 years. Thymidine kinase (TK) in serum was evaluated by TK REA and TK 'the novel technique' We analyzed correlation of serum TK with biochemical markers reflecting activity of multiple myeloma (MM), e. g. B2M, LDH, free light chains ratio: kappa/lambda K/L, infiltration of bone marrow by plasma cells (Pl. b). The imaging methods as MIBI or PET/CT which imagine active myeloma lesions were done at the time of diagnosis in 20 patients. **Results.** We found a strong positive correlation between TK values by TK REA and TK 'the novel technique'(r=0,822). After extreme values were excluded, the correlation coefficient r=0,787. There was clear correlation of serum thymidine kinase with biochemical markers reflecting activity of multiple myeloma (MM), e. g. B2M, LDH, free light chains ratio: kappa/lambda K/L, infiltration of bone marrow by plasma cells (Pl. b). Correlation TK 'the novel technique' vs. B2M, LDH, K/L, Pl. b. TK REA - positive correlation of medium strength with B2M (r=0,527) and LDH (r=0,553). TK 'the novel technique' - positive correlation of medium strength with B2M (r=0,456), LDH (r=0,573) and weak to positive correlation of medium strength with Pl. b. (r=0,386). Mann-Whitney U test showed at the group of patients with MM (stage I, II or III) significantly higher values of TK REA and TK 'the novel technique'in comparison to patients in stage MGUS. Median TK REA was 6,0 at MGUS, resp. 9,5 at MM (p=0,004), median TK 'the novel technique' was 27,6 at MGUS, resp. 135,9 at MM (p = 0,008). Imaging methods MIBI or PET/CT were positive in 13 patients, and negative in 7 patients. Increased levels of serum thymidine kinase were observed in MIBI or PET/CT positive patients, but correlation did not reached significance level. TK 'the novel technique' vs. PET/CT, MIBI (0=negative / 1 or 2 =positive) (without extreme values of TK 'the novel technique' Mann-Whitney U test did not show significant difference in values of TK REA or TK 'the novel technique' between group of patients with positive and negative PET, MIBI result (p=0,501, resp. p=0,285). AUC = 0,775 - it means TK 'the novel technique' could be used as a predictor for multiple myeloma. **Conclusions.** Statistical analysis revealed significant correlation between serum levels of thymidine kinase and LDH and B2M. Increased levels of serum thymidine kinase were observed in MIBI or PET/CT positive patients, but correlation did not reached significance level. **Supported by grant** *IGA MZ ČR NT 12215-4/2011.*

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A FOURIER TRANSFORM INFRARED SPECTROSCOPIC INVESTIGATIONS ON THE MOLECULAR PROFILING OF MULTIPLE MYELOMA

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Background. In the past several years, multiple recurring translocations,deletions and amplifications have been defined and mapped to the earliest stages of the developing MM clone. However gene expression profiling using SNPs have identified further abnormalities defining multiple clusters. Despite of all these efforts the molecular pathogenesis is still unclear. A complementary approach to the study of gene expression profiling is proteomic and molecular profiling. Fourier Transform Infrared (FT-IR) spectroscopy is a widely used and preferred method of infrared spectroscopy due to their speed and sensitivity for molecular profiling. With FT-IR, a complete molecular diversity of the samples(all types of organic and many types of inorganic compound) can be studied comparatively with a knowledge of the origins of the peaks(such as

lipids, proteins,carbohydrates,nucleic acids etc) and also the amount of the particular compounds,secondary-structures of proteins, post-translational modifications and protein-protein interactions can be determined. **Aims.** The aim of this study is to achieve molecular characterization of biomolecules in MM by using FT-IR spectroscopy and cluster analysis. **Methods.** Study group consisted of 6-MM, 4- monoclonal gammopathy of unknown significance (MGUS), 4-smoldering multiple myeloma(SMM) patients and 2-healthy controls who were selected from Ankara University Department of Hematology. Infrared spectra were obtained by Bruker-Tensor-27-FT-IR(Bruker-Optics,GmbH,Germany). Spectras from urine samples recorded in the mid-infrared-region, between 3800-850 cm^{-1} wave numbers and interferograms were accumulated for 32 scans at 4-cm^{-1} resolution at 22ºC in the single-bounce-ATR mode. All data collection, manipulation and analyses were performed by OPUS software(Bruker Optics). **Results.** According to our results, there are 9-significant peaks in FT-IR spectra of MM, SMM, MGUS and control samples (Figure 1A). It has been found out that absolute intensity of peaks were decreased in MM group compared to others groups. Second derivatives of absorbance spectra were calculated using Savitzky-Golay algorithm with 9-smoothing points. Relative intensity values of second derivative peaks in the Amid-I-region(1700-1590cm^{-1}) which were obtained by automated peak-picking were used to compare protein secondary structures (Figure-1C). Relative intensity of peaks in MM group were found to be decreased in 1680cm^{-1} and increased in 1650cm^{-1} compared to other groups (Figure-1D). These regions are known to represent C=O bonds in secondary structures of proteins. Cluster analysis(hierarchical clustering) was carried out by OPUS software and the results demonstrated two significant separation between MM, and SMM, MGUS and control groups (Figure-1B) and found to be clustered together as expected in normal diagnostic outcomes. Our preliminary findings show that FT-IR is feasible and replicable. During the congress comparison of findings between active-MM, SMM, MGUS and controls will be presented with protein profile datas. **Conclusions.** The current molecular technologies such as proteomics and molecular profiling are under development and is may be the reason why there is only one report on proteomics in MM(Micallef et al-2010). The advantages of overcoming the problem of uncoded genetic aberrations or inhibition by micro-RNAs and leading to direct measurement of proteins in quantity as well as demonstration of chemical structural changes may enhance the role of this technology in MM where secretory proteins and cytokines are essential elements.

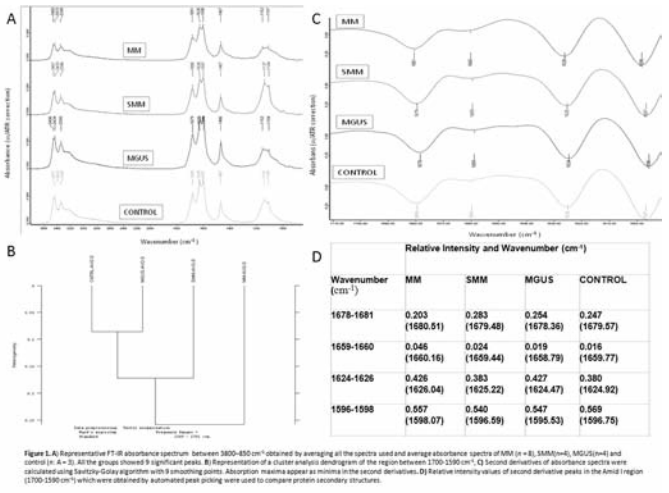

Figure 1. A) Representative FT-IR absorbance spectrum between 3800-850 cm^{-1} obtained by averaging all the spectra used and average absorbance spectra of MM (n = 8), SMM(n=8), MGUS(n=4) and control (n. 4 = 3). All the groups showed 9 significant peaks. B) Representation of a cluster analysis dendrogram of the region between 1700-1590 cm^{-1}. C) Second derivatives of absorbance spectra were calculated using Savitzky-Golay algorithm with 9 smoothing points. Absorption maxima appear as minima in the second derivatives. D) Relative intensity values of second derivative peaks in the Amid I region (1700-1590 cm^{-1}) which were obtained by automated peak picking were used to compare protein secondary structures.

Figure 1.

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EARLY B LYMPHOPOIESIS IN BONE MARROW IS SUPPRESSED WITH MULTIPLE MYELOMA PROGRESSION

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Background. Detailed analysis of lymphoid lineage subsets is helpful for evaluation of immune status. We previously described subpopulations of $CD34^+$ early lymphocyte progenitors in marrow that differ in CD10 expression. CD10 intensity correlated well with ability to proliferate in vitro and with lymphoid-lineage gene expression. In addition, $CD34^+$ $CD10^{Hi}$ and $CD34^+$ $CD10^{Low}$ populations had unique patterns depending on their sources; cord blood, bone marrow (BM) and G-CSF mobilized peripheral blood. We have now analyzed other B-lymphocyte precursors, collectively termed hematogones. Their numbers are known to vary in hematological malignancy, marrow regeneration and immunodeficiency. Our $CD34^+$ $CD10^+$ subpopulations in part overlapped with early stage hematogones. Although it might have clinical relevance, levels of hematogones in multiple myeloma (MM) patients are poorly understood due to the difficulty in distinguishing them from MM cells. **Aims.** To evaluate percentages of hematogones in MM patients using multi-color flow cytometry and to study the association with disease characteristics. **Methods.** BM samples were collected after informed consent, using protocols approved by the Investigational Review Board of Osaka University Hospital. Samples from patients with measurable MM cells, after an interval of more than 2 months from the last chemotherapy or 1. 5 years after high-dose chemotherapy with auto PBSCT, were immunophenotyped by 5-color (CD34/CD38/CD10/CD20/CD45) flow-cytometric analysis. Hematogones were divided into 4 maturation stages: $CD34^+$ $CD10^{Hi}$ stage 1, $CD34^-$ $CD10^{Hi}$ $CD20^-$ stage 2-1, $CD34^-$ $CD10^{Low}$ $CD20^{Low}$ stage 2-2 and $CD34^-$ $CD10^-$ $CD20^{Hi}$ stage 3. We sought correlations with age, percentages of MM cells in BM, albumin, β2microglobulin (B2M), creatinine and hemoglobin. *p* values < . 05 were considered to be significant. **Results.** There were 19 patients aged 52 to 78 years. Eleven were newly diagnosed, and 5 received high-dose chemotherapy following auto PBSCT. MM cells were clearly detected with CD38, CD20 and CD45 intensities, and percentages determined by flow cytometric analysis were consistent with those from microscopic evaluation. This isolation strategy made us possible to study normal lymphopoiesis in MM patients. Age, albumin, creatinine and hemoglobin showed no significant relevance. However, patients with high B2M (> 3. 5 mg/dl) had significantly lower percentages of stage 1, 2-1 and 3 hematogones, while mature lymphocyte counts in peripheral blood were not associated. The same was true for untreated patients. The burden of MM cells (>30% in BM) also affected the distribution among hematogone stages. Percentages of $CD34^+$ $CD38^-$ hematopoietic stem cells and $CD34^+$ $CD38^+$ hematopoietic progenitor cells did not show any correlation with B2M or tumor mass, indicating that the suppression of early hematopoiesis in MM is lymphoid lineage restricted. **Summary and Conclusions.** Most studies concerning impairments in cellular and humoral immunity in MM focused on regulation of mature lymphocyte function. We now show that early stages in normal B lymphopoiesis are suppressed in myeloma BM. In addition, there was a negative correlation between hematogones and the prognostic factor, B2M. Our study suggests that BM hematogone percentages may be useful for predicting MM patient outcome.

1491

PROTEASOME 20S CONCENTRATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background. The proteasome is a multicatalytic complex found in all eukaryotic cells. It is responsible for the degradation of many proteins which take part in cell development. During rapid cell turnover and growth proteasomes are released into the circulation and they can be measured in plasma by enzyme-linked immunoabsorbent assay (ELISA) techniques. Abnormally high level of proteasomes has been observed in leukemias, multiple myeloma and some solid tumors. Moreover, the concentration of circulating proteasome correlates with the disease state, and may have prognostic significance. **Aims.** The aim of our study was the assessment of the proteasome 20S concentration in the plasma of patients with newly diagnosed multiple myeloma (MM). **Materials and Methods.** The study included 30 patients with newly diagnosed MM (14 females, 16 men; mean age, 67. 3 years) and 15 healthy subjects (7 females, 8 men; mean age, 65. 7 years). The patients were followed-up in the Department of Hematology at the Medical University of Bialystok, Poland. All patients were divided into three groups based on international staging system (ISS): three patients at 1st stage, ten patients at 2nd stage and seventeen at 3rd stage. Approval for this study was obtained from the Local Ethic Committee. The concentration of proteasome 20S in the plasma was assayed using commercial test (20S/26S proteasome ELISA Kit, *Enzo Life Sciences)*. Statistical analysis was done using Shapiro-Wilk, Mann-Whitney and Spearman's tests. The statistical significance of the measured differences was determined using an alpha index of 0. 05. **Results.** Proteasome 20S concentration was significantly elevated in the patients with MM in comparison to healthy subjects (6. 63±4. 51µg/ml vs 3. 04±1. 34µg/ml, p=0. 004). Statistically significant correlations were found between proteasome 20S and albumin concentrations as well as between proteasome 20S concentration and serum LDH activity, but no correlation was found between proteasome 20S and β2-microglobulin concentrations (p=0. 64). The highest level of proteasome 20S was observed at 3rd stage of ISS score and the lowest at 1st stage. **Conclusions.** Our results confirm reports about increased concentrations of proteasome 20S in plasma of patients with

MM. The increased plasma concentration correlates with advancement of the disease.

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SERUM HSP70 PROTEIN AND ANTI HSP70 ANTIBODIES IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background. Multiple myeloma (MM) is the second most common hematologic malignancy that despite of great progress in its treatment, still remains incurable. Heat shock proteins (HSPs) are molecular chaperones involved in a number of cellular functions in stress conditions. It is well established that plasmatic cells express HSPs including HSP70. It has also been suggested that an increased surface expression of heat shock proteins on apoptotic tumour cells results in the generation of potent antitumour T-cell responses. On the other hand HSP70 may influence on sensitivity and resistance of MM cells for chemotherapy. Additionally, anti HSP70 antibodies are known to play a role in immunological and neoplastic processes. However their significance in MM has not been documented. **Aims.** The aim of our study was the assessment of the HSP70 protein and anti HSP70 antibody concentration in the MM patients. Materials and **Methods.** The studied group consists of 34 newly diagnosed, symptomatic MM patients (aged 41 -85), males 19 and females 15. The patients were in 1-3 stages of MM according to ISS scale. All of studied patients required chemotherapy. A control group contains of 20 healthy individuals. Identification and quantitative assessment of HSP70 antigen was performed using Western Blott method using specific antibodies (SPA810, Stressgen). Quantitative determination of anti-human HSP70 antibodies in the serum was done using commercial test (anti HSP70 Elisa Kits, Stressgen). The results are presented as mean ± SEM. Statistical analyses were carried out using software Statistica. The normality of continuous variables was assessed using the Shapiro-Wilk's test. As the continuous variables were not normally distributed, nonparametric statistical methods were used (Mann-Whitney and Spearman's tests). **Results.** Analysis of clinical samples showed the presence of HSP70 proteins in the serum of MM patients. The mean antigen concentration in MM group was slightly higher than in the control, 167,3±36,3 ng/ml and 107±8,1 ng/ml (p=0. 12) respectively. However, the level of antiHSP70 antibodies was significantly lower in the patient in comparison to healthy population (254,3±29,7 ng/ml vs. 489,1±95,3 ng/ml; p=0. 02). There was noticed no statistical significant decreasing in antiHSP70 antibody concentrations in more advanced stages of the disease according to ISS (333,1±134,3 ng/ml at 1st, 273,8±69,9 ng/ml at 2nd, 231,7±28,7 ng/ml at 3rd). The study showed no correlations between protein and antiHSP70 antibody concentration and other parameters such as age, gender and some prognostic factors (LDH, albumin, ß2-microglobulin). **Conclusions.** The results confirmed the presence of HSP proteins and antiHSP70 antibodies in serum of MM patients. The significantly lower serum level of antiHSP70 antibodies in the patients may reflect a participation of this type of heat shock proteins in MM pathogenesis. Futher studies are required to elucidate prognostic significance of HSP70 proteins and antiHSP70 antibody during the course of MM.

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MR-PROANP AND MCP-1 AS EFFECTIVE BIOMARKERS OF CARDIAC AND MICROVASCULAR INFLAMMATORY IMPAIRMENT IN SYSTEMIC AL AMYLOIDOSIS PATIENTS TREATED WITH MEL-DEX ASSOCIATION

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Background. Light-chain AL amyloidosis is the most common form of systemic amyloidosis and is associated with an underlying plasma cell dyscrasia. Circulation dysfunction is very frequent in AL amyloidosis patients, especially in those with a clinically-significant cardiac involvement, commonly resulting in moderate to severe heart failure depending on the extent and localization of amyloid deposits. MR-proANP (1-98), the stable mid-regional fragment of the active atrial natriuretic peptide prohormone (99-126), is widely considered an earlier predictive marker of cardiac impairment. In particular, it has direct correlation with NYHA functional class, so it's a powerful predictor of adverse outcome, especially if associated with elevated NT-proBNP values. MCP-1 chemokine plays a key role in the recruitment and activation of monocytes in sites of inflammation and it's actually considered an efficient marker in assessing the inflammatory activity in course of both multiple myeloma and systemic AL amyloidosis. **Aims and Methods.** Aim of the study is to investigate therapy induced modifications in circulation activity by MR-proANP and MCP-1 serum levels evaluation in patients with AL systemic amyloidosis. Blood samples were collected from 6 patients with systemic AL amyloidosis (median age 72. 8 yrs) admitted to our Unit and analyzed for serum MR-proANP (mean±SD) and MCP-1 levels (Kits Brahms MR-proANP Kryptor and Randox Evidence Biochips Arrays). According to age and disease risk stratification these patients were treated with upfront oral Mel-Dex association (Melphalan 9 mg/sm, Dexamethasone 20mg day 1-4 q28). From each patient 2 samples of peripheral blood were performed (T0 at exordium of disease, and T1 at conclusion of the first cycle of therapy). The blood was separated into plasma at the time of blood draw and frozen to -80°C. In the evaluation of results paired t test and Person correlation were performed. *P values ≤ 0. 05* were considered statistically significant. **Results.** MCP-1 levels were significantly higher in the sera of patients at the end of the first cycle of therapy (410. 10 ± 159. 70pg/ml) compared to exordium (333. 20 ± 148. 80 pg/ml; *p=0. 04*). MR-proANP serum levels were significantly decreased (141. 60 ± 45. 48 pmol/L) respect to baseline (183. 2 ± 76. 23 pmol/L; *p=0. 038, see Figure 1*). No positive correlation has been found between the two parameters. **Conclusions.** The high MCP-1 serum levels observed at the end of therapy can be expression of a persistent and increasing inflammatory activity during treatment. On the other hand, MR-proANP serum level reduction could suggest a coming moderate improvement of heart function, not clinically relevant at once. On the basis of our results, MR-proANP and MCP-1 can be used as effective biomarkers to understand the cardiac and microvascular inflammatory processes and their modifications in treated patients with systemic AL amyloidosis.

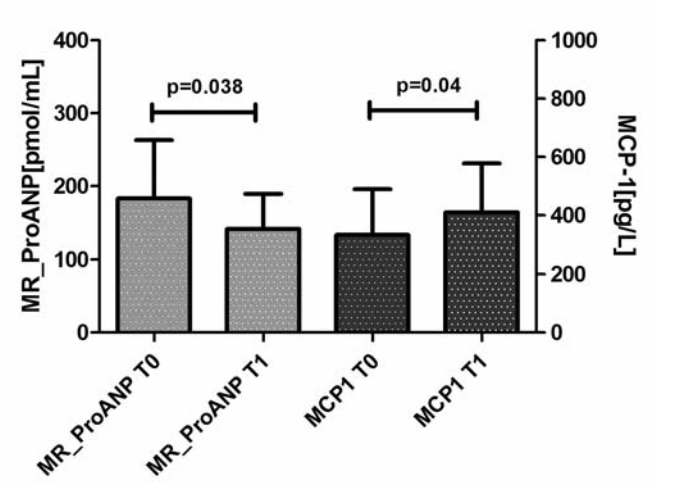

Figure 1.

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CHROMOSOMAL CHANGES IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES: LINKS TO FAMILIAL PARAPROTEINEMIA

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Background. Monoclonal Gammopathy (MG) is caused by expansion of a single clone of B-lymphocytes. MG can be pre-malignant (MGUS) or malignant (multiple myeloma (MM), Waldenström's macroglobulinemia (WM)). The immunoglobulin produced can be of IgM, IgG or IgA type, depending on whether the clonal expansion has taken place after class switching. The incidence of MG increases with age and the probability of progression from MGUS to malignancy is about 1-1. 5% each year. IgM-MGUS is more likely to become WM but IgG- or IgA-MGUS can lead to MM. Families with multiple MG cases fall into these two categories, indicating different genetic backgrounds. Some

genomic changes in the progression from MGUS to malignancy have been identified. Translocations involving the IgH locus on chromosome 14q32. 3 and genomic instability, including chromosome losses are prevalent in MM but not WM. Little is known about genomic changes that lead to MGUS. More than 130 families worldwide with multiple cases of MGUS, with or without MM or WM have been reported. Based on published information, first degree relatives of MGUS patients have a three fold risk of developing MM and a two-fold risk of developing WM. An Icelandic family with high prevalence of MG was first reported in 1978. One third of healthy individuals in this family had hyper-responsive B-cells, producing significantly more Ig than B-cells from controls following mitogen-stimulation *in vitro*. The hyper-responsive B-cells survived over longer time in culture and showed prolonged expression of Bcl-2. Seven more families have been found in Iceland with high prevalence of MG and in three of these hyper- responders (HR) were identified. All four families with HR contain cases of both MM and WM. **Aims.** The aim of this study was to screen for chromosomal translocations and smaller genetic changes in peripheral blood B-cells from HR, related and unrelated controls. **Methods.** Breaks on chromosome 14q32. 3 were detected by interphase-FISH (Fluoricence *in situ* hybridization). Array-CGH (Comparative Genome Hybridization) was performed on isolated DNA from each person, screening for loss or gain in B-cells DNA vs. granulocytes by using 135. 000 probes throughout the genome. Conventional cytogenetic analysis was done on metaphases after culture for 7 or 14 days in an *in vitro* model of the germinal centre reaction. **Results.** FISH and conventional cytogenetic analysis did not show any significant differences between HR and controls. CGH analysis detected changes in Ig-genes in B-cells as expected. In addition, all B-cell samples showed marked gains and losses throughout the genome, presumably reflecting random effects of hypermutations during the germinal centre reaction. Significantly fewer gains were detected in HR vs. controls on 7 chromosomes. **Conclusions.** These results are consistent with a tendency towards developing WM rather than MM, with no evidence of excess IgH translocations but indications of relatively low germinal centre activity.

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OSTEOGENIC DIFFERENTIATION POTENTIAL OF HEALTY AND MULTIPLE MYELOMA BONE MARROW MESENCHYMAL STEM CELLS

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Background. Multiple myeloma(MM) is a plasma cell malignancy that cause osteolytic bone lessions. In healty bone marrow osteoblast formation and differentiation from mesenchymal stem cells called bone formation and bone resorbtion by osteoclastic activity are in a balance. MM cells induce an imbalance between osteoblastic and osteoclastic activity by producing many inhibitory factors. Studies have shown that MM patients may have a reduction of bone formation markers such as alkaline phosphatase(ALP). **Aims.** In our study we examined osteogenic differentiation potential of mesenchymal stem cells derived from bone marrow (BM-MSCs) of MM patients and healty donors. **Methods.** Three patients with MM and 3 healty donors' BM-MSCs were obtained and cultured in appropriate conditions. At passage 3 after they characterized by flow cytometry and treated with osteogenic stimulatory medium for 30 days. The ALP activity assay were performed on the cells at 1.,3.,7.,14. and 21 was normalized by total protein concentration. In addition, at the end of 4 weeks osteogenic differentiation was assessed by staining with bone formation markers such as Alizarin Red S. The relative gene expressions of BMP-2, BMP-4 and ALPL were performed by Real time PCR. **Results.** The results demonstrate that in the 3. day of differentiation, the ALP activity started to increase in healty donors. But in MM there is no ALP activity up to 21 days. In Alizarin red S staining there were a few minor calcium nodules compared with healty donors. We compared gene expressions with healty donors and MM MSCs and the levels of these genes (BMP-2, BMP-4 and ALPL) were significantly reduced in MM-MSCs. **Conclusions.** Our data have shown that the reduction of expression of osteogenic genes in MM BM-MSCs and defect of ALP activity may be related with osteogenic disorders of MM in addition to presence of other tumorogenic microenviroment of bone marrow.

1496

HYPERMETHYLATION OF TUMOR SUPPRESSOR GENES IN KOREAN PATIENTS WITH MULTIPLE MYELOMA

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Background. Aberrant DNA methylation is involved in the initiation and progression of carcinogenesis and includes hypermethylation of CpG islands of tumor suppressor gene promoters. Cyclin-dependent kinase 2A (*CDKN2A*), O^6-methylguanine DNA methyltransferase (*MGMT*), and E-cadherin (*CDH1*) are known to be hypermethylated in various neoplasms. **Aims.** We aimed to investigate the methylation status of tumor suppressor genes using pyrosequencing in Korean patients with multiple myeloma (MM). **Methods.** Three cell lines, ARH77, RPMI8226, and MC/CAR, and bone marrow aspirates from 10 patients with MM were analyzed by pyrosequencing. All the patients had given written informed consent. The methylation index (MtI) of each gene promoter was calculated as the average value of methylated cytosine [mC/(mC+C)]. Hypermethylation was defined as MtIs of *CDKN2A*>0. 047, *MGMT*>0. 053, *CDH1*>0. 081. **Results.** CpG island hypermethylation of promoters of *CDKN2A*, *MGMT*, and *CDH1* were found in all the MM cell lines. Mean MtIs of *CDKN2A*, *MGMT*, and *CDH1* in cell lines were 0. 937, 0. 250, and 0. 576, respectively. Hypermethylation of *CDKN2A*, *MGMT*, and *CDH1* were detected in 80% (8/10), 56% (5/9), and 89% (8/9) of the MM patients, respectively. Mean MtIs of *CDKN2A*, *MGMT*, and *CDH1* in the MM patients were 0. 108, 0. 093, and 0. 243, respectively, which were significantly higher than control group (P<0. 05). **Summary / Conclusions.** Our results suggest that hypermethylation of tumor suppressor genes (*CDKN2A*, *MGMT*, and *CDH1*) are frequent events in MM, which may an important role in the pathogenesis of MM. Pyrosequencing is useful for detection of methylation and it offers quantitative data and high throughput.

1497

VALPROIC ACID UPREGULATES NKG2D LIGAND EXPRESSION AND POTENTIALLY ENHANCES NATURAL KILLER CELL-MEDIATED CYTOLYSIS OF MYELOMA

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Objective. Although high-dose chemotherapy supported by autologous hematopoietic stem-cell transplantation can produce higher response rates than standard chemotherapy, multiple myeloma (MM) remains largely incurable by current therapeutic strategies. Clinical and preclinical data have been demonstrated that natural killer (NK) cells have an anti-myeloma effect. However, low NK cell activity affected the successful utilization of NK cell adoptive immunotherapy for myeloma. In this study, we investigated the effect of valproic acid (VPA), as a histone deacetylase inhibitor, on the expression of NKG2D ligands on human myeloma cell lines and primary myeloma cells derived from patients. We also evaluated the sensitivity of myeloma cells to NK cell-mediated lysis before and after treatment with VPA, and the possible underlying mechanisms. **Methods.** ARK, OPM2 and primary myeloma cells (n = 4) from patients with myeloma were cultured with VPA for 24 to 72 hours. To evaluate the effect of VPA on NKG2D ligand expression of myeloma cells, mRNA and protein expression of NKG2D ligands of myeloma cells were tested by Real time-PCR and flow cytometry. *51Cr release assay and* NKG2D antibody blocking experiments *were* combined to examine the susceptibility of myeloma cells to NK cell-mediated lysis in response to VPA and the possible killing mechanisms. **Results.** ARK and OPM-2 were treated with various concentrations of VPA. The most prominent upregulations of mRNA expressions of MICA ULBP2 and ULBP 3 were observed in the two tested myeloma cell lines. The enhancement of cell surface expression of MIC ULBP2 and ULBP 3 was detected by flow cytometry after cells treatment with VPA. A similar increase of surface expression of NKG2D ligands was obtained in VPA treated primary MM cells (n=4). VPA treatment of OPM2 showed higher sensitivity to NK cell lysis than untreated cells (specific killing: 72. 8±6. 2% vs 32. 1±3. 7% at E/T 10:1, p <0. 01). The killing of VPA treatment of patient myeloma cells by NK cells also significantly increased compared with control cells (specific killing: 46. 7±5. 6% vs 21. 6±4. 5% at E/T 10:1, n=4, p <0. 01). The enhancing effect of VPA was blocked by pretreatment of OPM2 cells with a combination of anti-MICA/B, anti-ULBP1,2,3 mAbs or pretreatment of NK cells with anti-NKG2D mAbs, indicating the contribution of NKG2D-NKG2D ligands interations to VPA-modulated lysis of myeloma. We also found that constitutive phosphorylated extracellular signal-regulated kinase (ERK) but not AKT signaling pathway may be involved in VPA-induced higher NKG2D ligand expression. **Conclusions.** Histone deacetylase inhibitor valproic acid could upregulate cell surface expression of NKG2D ligands, rendering myeloma cells more sensitive to NK cells, and thereby enhance NK cell-mediated lysis of myeloma. Our findings imply that regulation of the expression of NKG2D ligands by treatment with histone deacetylase inhibitors may be an attractive strategy for immunotherapy of myeloma. **Acknowledgments:** This work was supported by National Natural Science Foundation of China (81071856 and 30973450), Shanghai Pujiang Program (11PJ1407900) and Shanghai Tenth People's Hospital (10RD103 and 11SC103).

1498

IMPACT OF INCIDENCE GAIN(1)(Q21) FOR RELAPSED MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE OR BORTEZOMIB-BASED REGIMENS

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Prognostic impact of specific chromosomal aberrations in relapsed multiple myeloma (MM) patients treated with the novel agents is briefly described. We analyzed the prognostic value of extended panel of chromosomal aberrations (del(13)(q14), del(17)(p13), t(4;14)(p16;q32), gain(1)(q21) and hyperdiploidy using I-FISH technique in a cohort of 127 relapsed MM patients treated with thalidomide or bortezomib-based protocols. In the thalidomide group, we found significant difference in overall survival (OS) between group of patients with and without gain(1)(q21)(15. 7 versus 41. 3 months; P=0. 004). We confirmed negative impact of cumulative effect of two or more cytogenetic changes occurring simultaneously on OS in the thalidomide group (20. 3 months vs. not yet reached; P=0. 039). We did not find any significant impact of studied aberrations on overall survival in the bortezomib cohort of patients. We conclude that bortezomib-based protocols are able to overcome the negative prognostic impact of tested chromosomal abnormalities in relapsed MM patients

1499

TUMOR GROWTH IS ASSOCIATED WITH INCREASED ANGIOPOIETIN-2 SERUM LEVELS IN MULTIPLE MYELOMA PATIENTS

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Background. Angiogenesis has been found to be an essential component in the growth and progression of multiple myeloma (MM). Angiopoietins (Ang) are growth factors that play a critical role in the regulation of vessel formation. Among them, Ang-2 and its receptor Tie-2 appear to play an important role in tumor angiogenesis. **Aims.** The aim of the current study was to determine serum levels of circulating Ang-2 and its receptor Tie-2, in newly diagnosed MM patients and to estimate if there is any relationship between them and known markers of disease severity, such as C-reactive protein (CRP), beta-2 microglobulin (beta-2m), lactate dehydrogenase (LDH) and interleukin-6 (IL-6) serum levels as well as M-component value. **Methods.** We studied 55 newly diagnosed myeloma patients (29 male, 26 female, with mean age 66. 9±13. 7 years). According to Durie-Salmon staging system 8 had stage I disease, 25 stage II and 22 stage III. We also studied 30 of them in plateau phase, as well as 20 healthy controls. Ang-2, Tie-2 and IL-6 serum levels were determined by ELISA, using commercially available kits. The remaining variables were determined using standard laboratory methods. **Results.** Ang-2, IL-6, CRP, beta-2m and LDH serum levels, as well as M-component values, were higher in MM patients compared to controls (p<0. 001 in all cases). Ang-2 serum levels were also higher in advancing disease stages (p<0. 001) as well as in pre-treatment group compared to plateau phase (p<0. 001). On the contrary, Tie-2 serum levels did not show any significant difference in either case. We also found significant correlations between Ang-2 serum levels with CRP (r=0. 469 p<0. 001), beta-2m (r=0. 577 p<0. 001), LDH (r=696, p<0. 001) and IL-6 (r=0. 623 p<0. 001) serum levels. On the other hand, none significant correlation was found between Tie-2 serum levels and either marker of disease severity. **Conclusions.** Our results showed that Ang-2 serum levels, which reflect bone marrow neovascularization, are increased in MM patients and correlated with known markers of disease severity, such as beta-2m, IL-6, LDH and CRP. This finding indicates that Ang-2 could be used as a potential tumor marker for disease severity, as well as a possible target for MM treatment.

1500

EVALUATION OF METALLOPROTEINASES EXPRESSION LEVELS IN MGUS AND MM PATIENTS

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Matrix metalloproteinases (MMPs) are a family of structurally and functionally related proteinases characterized by the ability to degrade the extracellular matrix (ECM). Based on their substrate specificity and domain structure, MMPs were divided into subgroups. One of these subgroups is represented by gelatinases, which degrade gelatine and several types of collagen, like gelatinase A (MMP-2) and gelatinase B (MMP-9). MMPs are known to play a role in cell growth, invasion, angiogenesis, metastasis, and bone degradation, all important events in the pathogenesis of cancer. Multiple myeloma is a B-cell cancer characterized by the proliferation of malignant plasma cells in the bone marrow, increased angiogenesis, and the development of osteolytic bone disease. The first pathogenic step is a premalignant monoclonal gamopathy of undetermined significance (MGUS). In the progression from MGUS to MM, complex genetic events occur in the neoplastic plasma cell (PC), and in the bone marrow (BM) microenvironment, including induction of angiogenesis, suppression of cell-mediated immunity, and development of paracrine signalling loops involving interleukin-6, insulin-like growth factor 1, interferon α and vascular endothelial growth factor. Furthermore, although tumour progression is observed mainly within the bone marrow during the early stages of the disease, extramedullary spreading occurs during the terminal stage of the disease. Moreover, malignant cells can be detected in peripheral blood of many patients with MM, suggesting migration of myeloma cells outside the bone marrow. However, the role of MMPs in the development of MM is poorly understood. In the present study, we aimed to explore the role of MMPs, namely MMP-2, MMP-8 and MMP-9, in the pathogenesis of MGUS and progression to MM. A total of newly diagnosed 16 MGUS patients and 16 MM patients and 2 healthy individuals (controls) were included in this study. Expression of MMP-2, MMP-8 and MMP-9 was assessed on bone marrow plasma cells (PC) by flow cytometry using a four-color staining assay. The average MGUS patients' age is 72 years (42-90), 62,5% female and 37,5% male. The MM patients were 62% female and 38% male, with an average of age of 78 years (67-87). Our preliminary study shows that MGUS and MM PC patients have higher MMP expression levels compared with controls. On the other hand, MM patients show higher percentage of PC expressing MMP-9 when compared to MGUS patients. However, in $CD19^+/CD138^+$ PC the intracellular MMP expression levels are higher in MGUS patients when compared to MM, while in malignant PC ($CD19^-/CD138^+$) these differences are mainly observed in MMP-9. When analysed both PC patient population, we observed in $CD19^+/CD138^+$ PC an increase in MMP-2 and -8 expression levels and in the percentage of cells expressing these MMP compared with PC $CD19^-/CD138^+$. Besides that all MM patients are positive for at least to MMPs. Our findings suggest that PC MMP expression may be correlated with transition of MGUS to MM, promoting extramedullary spreading and disease evolution. Since MM remains incurable, confirmation of these results may contribute to a better understanding of MM biology and can lead to new therapeutic approaches.

1501

LEVELS OF FREE LIGHT CHAINS IN THE SERUM OF NORMAL SUBJECTS AND PATIENTS WITH MONOCLONAL GAMMOPATHY

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Background. Monoclonal gammopathy (MG) is defined as a plasma cell lymphoproliferative disorder characterized by the production of a monoclonal immunoglobulin with expression of a single type of light chain. Monoclonal component (MC) detection in serum is important for diagnosis and monitoring of MG. **Aims.** To study the free light chains (FLCs) and the ratio kappa/Lambda in the serum of patients with a monoclonal component detected by serum protein electrophoresis (SPE). To evaluate their clinical utility as a biochemical marker for screening, diagnosis and monitoring of MG. **Methods.** Serum samples from 125 healthy subjects, 51-79 years of age, with estimated ratio of glomerular filtration (GFR) ≥90 mL/min and normal protein electrophoretic profile without detectable monoclonal component. We obtained serum samples from 104 patients and we detected monoclonal band by means of SPE and Immunotyping (IT): 68 samples were from patients with a monoclonal kappa light chain and 36 from patients with a monoclonal lambda light chain. The patients were between 57-78 years old, 59 of whom

were women and 45 men. SPE was performed by automated assay (Capillarys protein/Sebia) and calculated the MC (g/dL). IT was performed with a Capillarys Immunotyping/Sebia. FLCs were quantified with Freelite$^{TM)}$ reagent from The Binding Site and nephelometry on a Dade-Behring calculating kappa/lambda ratio. We estimate the glomerular filtration ratio by the Cockcroff-Gault formula. We used the statistical software SSPS to the compare the means of the patients and the control group. We calculate the correlation coefficient between the concentrations of MC (g/dL) and the concentration of FLCs (mg/dL). **Results.** In the control group FLCs of free kappa light chain ranges from 0. 6 to 1. 92 mg/dL (mean 1. 26 ± 0. 33), the free light chain lambda ranges from 0. 43 to 1. 8 mg/dL (mean 1. 14 ± 0. 33), the ratio k: L ranges from 0. 8 to 1. 48 (mean 1. 14 ± 0. 17). We have found a significant increase of FLCs in the pathological groups when compared to the reference group (p<0,001) and a positive correlation between the concentrations of MC and FCLs (r = 0. 81 and r = 0. 84 respectively). The ratio kappa/lambda is altered in all patients with MG (being higher than 1. 65 in MG with excess kappa FLC and lower than 0. 26 in MG with an excess of lambda FLC). **Conclusions.** The ratio kappa/lambda is an effective marker of clonality because there is complete consistency between the ratio K/L and the results of IT. In MG associated with FLCs, these proteins may be present in serum in small amounts and thus be difficult to detected by IT and often impossible to quantify on SPE. The incorporation of the determination of serum free light chains to the study of MG is recommended as an aid to diagnosis, prognosis and monitoring of patients.

1502

TRAIL EFFECT ON OSTEOCLAST FORMATION IN MULTIPLE MYELOMA-BONE DISEASE

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In physiological conditions OCs differentiation is finely controlled by macrophage colony stimulating factor (MCSF) and receptor activator factor of nuclear factor kappaB ligand (RANKL). In pathological conditions (<i*e.* bone resorptive diseases) several cytokines and growth factors are directely or indirectely implicated in osteoclastogenesis modulation. Among others, the tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL), belonging to the TNF superfamily of cytokines, seems to be implicated in OCs differentiation. However there are contradictory literature data on TRAIL effects on osteoclastogenesis process. Here, we investigated the effect of TRAIL on OC formation both in physiological conditions and multiple myeloma(MM)-bone disease by means of an osteoclastogenesis in vitro model consisting of peripheral blood mononuclear cells (PBMCs) obtained from healthy donors or patients with MM-bone disease. In particular the samples included peripheral blood (PB) from 32 MM patients at diagnosis (15 males and 17 females, median age 65. 5 +/- 8. 7) and 30 control subjects matched for age and sex with MM patients. In PBMCs from healthy donors, we demonstrated that TRAIL can directly induce OC formation, whereas an inhibitory effect on OC formation was observed when TRAIL was added with RANKL concomitantly. The levels of TRAIL were detected in the sera and in the media of PBMC cultures from the patients as well as from the same samples of control subjects using a human TRAIL enzyme-linked immunosorbent assay (ELISA) kit (supplied by Biomol Research Laboratories, Inc., Plymouth Meeting, PA, USA). In the culture media of PBMCs from the patients we detected high levels of TRAIL. To better understand the role of TRAIL in OC formation in MM-bone disease, we performed osteoclastogenesis experiments by culturing PBMCs from patients in the presence of different concentrations of a neutralizing anti-TRAIL mAb. Interestingly, the anti-TRAIL dose-dependent inhibition of spontaneous osteoclastogenesis was completely abolished by the RANKL addition in the same culture system. Our results suggest that, in an *in vitro* system, TRAIL and RANKL show different behaviour in MM-bone disease and normal conditions.

1503

CHROMOSOME 1Q GAIN IS A COMMON ADDITIONAL GENETIC ABERRATION IN RELAPSED MYELOMA PATIENTS

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Background. Multiple myeloma is a neoplasm of the antibody producing plasma cell. All myeloma cells have genetic aberrations. Primary immunoglobulin translocations and hyperdiploidy are all present in the premalignant condition MGUS. This is not the case with other common aberrations like deletion 13q and 17p, which usually increase in incidence and percentage of affected cells during disease progression. **Aims.** We had sequential samples from ten myeloma patients collected between 2006 and 2011 and wanted to find out if there were any genetic aberrational changes in the patient samples during disease progression. **Methods.** We used interphase fluorescence in situ hybridization to detect genetic aberrations. The probes covered the following loci: 14q32, 11q13, 4p16, 16q23, 6p21, 20q12, 8q24, 13q14, 2p11, 22q 17p, 1p and 1q21. 100 nuclei were scored for each sample. **Results.** For two of the patients we had three sequential samples, for the rest two samples. The most prominent finding was the gain of chromosome 1q21 in 6 of the patients after relapse. Patients with gain of chromosome 1q21 at diagnosis had both an increased copy number of 1q, and increased percentage of cells affected of the aberration. From one of the patients we were able to establish a cell line, URVIN. This cell line have a t(4;14). At diagnosis we found 54% deletion 13, normal chromosome 17 and amplification of chromosome 1q21. At relapse we found 86% deletion 13q, 68% deletion 17p and 87% deletion 1p. 1q21 was heterogeneous with 30% 3 copies, 29% 4 copies and 30% 5 copies. In the cell line, URVIN, all cells had deletion 13q, deletion 17p, deletion 1p and 3 copies of 1q. **Summary and Conclusions.** In our material, chromosome 1q amplification was the most common additional genetic aberration in relapsed myeloma patients.

1504

SERUM FREE LIGHT CHAINS AND SERUM IMMUNOGLOBULIN SPECIFIC HEAVY/LIGHT CHAIN PAIRS ALLOWS IDENTIFICATION OF SPECIFIC CLONAL PRODUCTION

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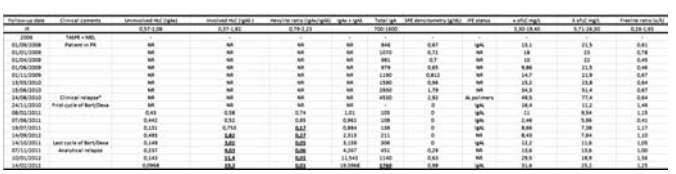
Background. A novel polyclonal immunoassay specific for the different light chain types of intact immunoglobulins (Igs) (heavy/light chain assays (HLC) together with the serum free light chain (FLC) test enables the measurement of changes in the production of clone specific Igs. By calculating the HLC ratio, the relationship between clonal and non-clonal plasma cells can be assessed. Increasing evidence indicates progressive clonal evolution and recently has been demonstrated the prognostic value of the HLC ratio together with sFLC ratios. A two tiered prognostic model employing the HLC and FLC tests have been proposed, and the use of these assays to analyze the clonal tide in patients with MM is becoming a realityCase presentation: A 66 year old man previously diagnosed in 1996 with a smoldering multiple myeloma (SMM) IgA lambda progressed to an IgA lambda multiple myeloma (MM) stage IIA in November 2004. At this time point patient presented normal cytogenetic. In September 2005 patient was treated with VMCP/VBAD (6 cycles) plus pamidronate achieving a partial response (PR). In 2006, patient presented a bone marrow infiltration of 20% with no osteolitic lesions and in June 2006 underwent a STEM cells -transplant achieving again a stable PR until August 2010. In August 2010 the patient suffered a clinical relapse suffering from acute back pain as result from a pathological fracture located in D10 and S2 vertebral body. M-spike was 2,92 mg/dL and sIFE presented different polymers if IgA. However, since patient first treatment, sFLC ratios were normal all the time contrasting with the results obtained with SPE and sIFE. After the second relapse the patient was treated with bortezomib/dexametasone with last cycle being applied at the end of October 2010. Interestingly, sFLC ratios were normal since the first PR (until the present day). In November 2011 a new "analytical"relapsed was observed by SPE and HLC but sFLC remains stable. The measurements of the uninvolved HLC were always immunosuppressed. The analytical follow-up is presented in the Table 1.

Table 1.



Conclusions. Last evidences indicates the possibility of diferent clones being envolved in diferent stages of the disease. Espeacialy nowadays, because of the use of powerfull and efective new agents, clonal tiding can be seen at diferent time points and sometimes diferent relapses can occur due to diferent expression of diferent clones. In these cases, sFLC together with HLC ratios could help us to distinguish these diferent clones and could orientate us in the future treat-

ment options. On the other side, due to the high sensitivity of HLC assays, the last relapse was detected aproximatly 3 months before SPE by showing a steadly increase of the monoclonal HLC pair and altered ratios.

1505

MULTIDRUG RESISTANCE 1 (MDR1) GENE AND ABC-FAMILY PROTEINS (MDR1 AND MRP1) IN MONCLONAL GAMMOPATHIES (MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNCERTAIN SIGNIFICANCE)

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Background. Multiple myeloma (MM) is consistently preceded by a premalignant step, the monoclonal gammopathy of uncertain significance (MGUS). In the progression from MGUS to MM, complex genetic events occur in the neoplastic plasma cell (PC) and in the bone marrow (BM) microenvironment. A major limitation in the treatment of MM patients is the development of multidrug resistance (MDR) and a well-known responsible mechanism is the overexpression of ABC-transporter genes such as MDR1. P-glycoprotein (P-gp), encoded by MDR1 gene, is an ABC transporter involved in the efflux of a variety of anti-cancer drugs out of the cell and in the cross-resistance to structurally unrelated cytotoxic agents. MDR1 gene polymorphisms (C3435T and CYP3A4) could alter P-gp expression levels resulting in drug resistance. The MDR1 C3435T polymorphism was previously described to be involved in immunological response. Furthermore, a drug efflux-independent mechanism of drug resistance seems to be mediated by P-gp, namely the transport of phospholipids across the cell membrane, contributing to its antiapoptotic function. Other transporter proteins mediating drug resistance include the multidrug-resistance-associated protein (MRP) family, notably MRP1. Besides recent studies have indicated that specific mechanisms of MDR may be involved in refractory MM patients, the expressions of ABC family members have not been well worked out in MGUS and MM patients. **Aims.** To analyze the expression of ATP-binding cassette proteins, MDR1 and MRP1, in patients with monoclonal gammopathies (MGUS and MM) and to evaluate the involvement and prognostic role of MDR1 C3435T and CYP3A4 T673C polymorphisms in the development of monoclonal gammopathy and in the progression from MGUS to MM. **Methods.** For this purpose, 47 patients with monoclonal gammopathy (23 symptomatic untreated MM patients and 24 MGUS patients) and 51 healthy individuals (controls) were enrolled in the study. The expression of MDR1 and MRP1 proteins in plasma cells was performed by flow cytometry using monoclonal antibodies labeled with fluorescent probes. PCR-RFLP was used for the detection of C3435T and T673C polymorphisms, using the restriction enzymes MBOI (MDR1) and Alw261 (CYP3A4), respectively. Group comparisons were evaluated with the c2, Fisher's exact test and t-student test.

1506

NEUROLOGICAL MONITORING DURING BORTEZOMIB AND/OR IMIDS REDUCE THE DEVELOPMENT OF SEVERE PERIPHERAL NEUROPATHY IN MULTIPLE MYELOMA PATIENTS: A SINGLE CENTER EXPERIENCE

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Background. Dose-limiting peripheral neuropathy (PN) has been reported in up to 75% of multiple myeloma (MM) patients treated with Bortezomib and/or IMIDs, requiring dose reduction, delay or interruption of treatment. Careful neurological monitoring and early diagnosis of PN may prevent irreversible neurological damage. **Aims.** We performed a monocentric prospective study to investigate the possible benefit of regular neurological assessment in reducing the development of severe PN. **Methods.** All patients were evaluated before, during and after MM treatment by clinical and neurophysiologic assessment. The neuropathy was graded in levels 1 to 4 according to the National Cancer Institute-Common Toxicity Criteria version 3 [NCI-CTC]. Neuropathic pain intensity was expressed according to the patient-reported Numerical Rating Scale [NRS]. **Results.** Between March 2007 and June 2011, 100 consecutive patients were included. The clinical characteristics were: median age 59 years (32-77); 46 men and 54 women; 42 and 58 pts were, respectively, in Durie and Salmon stages I-II and III. Seventy-five patients received >2 lines of treatment (range 1-7). Sixty-three patients were treated with bortezomib, 31 with thalidomide, 25 with bortezomib-thalidomide combination and 51 with lenalidomide. After a median time of 22 months (range 1-171) from the start of treatment, 65 patients developed PN (56 sensitive, 9 sensitive-motory), 31 during first line therapy, 29 during second line and 5 after the second line of therapy. At the time of PN diagnosis, 26 patients were receiving bortezomib, 15 thalidomide, 20 the bortezomib-thalidomide association and 4 lenalidomide. In 2 cases, PN was diagnosed before the start of treatment. The median time to PN diagnosis was 2. 8 months for bortezomib, 10. 5 months for thalidomide and 2 months for bortezomib-thalidomide. There was no significant correlation between PN occurrence and cumulative dose of bortezomib (median 30 mg) and thalidomide (median 9. 900 mg). According to the NCI-CTC toxicity criteria, 25 patients developed grade 1, 34 grade 2, 6 grade 3, 0 grade 4 PN. Neuropathic pain occurred in 30 patients; the median NRS was 5. 5 (range 4-9) and it was treated with opioids and tricyclic antidepressants. After PN diagnosis, 18 patients required bortezomib/thalidomide dose reduction, 3 patients a temporary drug discontinuation and 5 patients treatment interruption, while 39 patients continued their treatment with close neurological assessment. With a median follow up of 56. 5 months (range 7-232), 28 patients showed PN resolution. In 6 cases, PN worsened during bortezomib retreatment. **Conclusions.** Our study shows that clinical and electrophysiological examination are useful in the early diagnosis of PN. Careful monitoring is required to guide the physician in dose adjustment, especially when bortezomib and thalidomide are used in combination, in order to avoid the development of grade 3-4 PN. With accurate neurologic monitoring only 7% of patients had stop treatment. Also the use of oral opioids and tricyclic antidepressants seems to be effective in reducing the intensity of the neuropathic pain, as well as the impact of this symptom on the patient's quality of life.

1507

EFFICACY OF BORTEZOMIB-BASED THERAPY IN RELAPSED/ REFRACTORY MULTIPLE MYELOMA: Results FROM AN ELECTRONIC BORTEZOMIB OBSERVATIONAL STUDY (EVOBS) FOR PATIENT ENROLLED IN RUSSIAN FEDERATION

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Background. The international, non-interventional electronic VELCADE observational study (eVOBS) is ongoing to prospectively evaluate the efficacy and clinical outcomes associated with bortezomib-based (BB) therapies for primarily relapsed/refractory multiple myeloma (MM) in the 'real-world' clinical practice setting. Here we report data of patient fromRussian Federation which were summarized and separated from entire population enrolled into the eVOBS study. **Aims.** With study enrollment now complete, we report patient demographics, baseline disease characteristics, and efficacy data for Russian eVOBS study population. **Methods.** Adult patients scheduled to begin BB therapy for the treatment of predominantly relapsed/refractory MM were eligible for enrollment into the study. Patients were enrolled at clinical practices and provided written informed consent which was obtained in accordance with the Declaration of Helsinki. Treatment history was retrospectively documented for 1 year prior to initiation of BB therapy. Prospective observational data were collected for up to 3 years after initiation of therapy to document efficacy data. All administered bortezomib doses and concomitant treatments (except for investigational therapies) were permitted. MM disease stage was assessed at the time of initiation of BB therapy using Durie-Salmon (DS) staging system. Responses were investigator-assessed and based on European Group for Blood and Marrow Transplantation, or M-protein criteria. Response data were censored at the start of subsequent therapy, and for deaths. **Results.** Total of 691 patients were enrolled between October 2006 and December 2010 (40% male, 60% female, median age 59. 6 years). The patients had stage II (42%) or stage III (51%) of the disease according DS system prior to treatment initiation. The median time from diagnosis to treatment initiation was 2. 0 years; the median time from the start of primary therapy to enrollment was 1. 8 years. The majority of patients (97%) included in Russia received BB therapy for relapsed/refractory MM; 3% of patients received bortezomib as their first-line therapy. The most common prior MM treatments documented within 1 year of starting BB therapy were melphalan-prednisone combination (29%), vincristine-doxorubicin-dexamethasone combination (9%), other primary and second-line regimens (7%); and 55% had no MM treatment in the previous year. 43% of patients received monotherapy with bortezomib, 36% - in combination with dexamethasone, 15% - with prednisone, and 6% - with other agents including thalidomide, lenalidomide . Most of patients (85%) received bortezomib at an initial dose of 1. 3 mg/m^2. At the data 86. 1% (overall response rate) of patients achieved a best response: complete response (33. 9%), near-CR (21. 0%), partial response (26. 8%), and minimal response (4. 4%). At the data cut-off (39 months from the study initiation) the median survival was not reached. The median progression-free survival time was 2. 9 years. 461/691 patients (66. 7%) discontinued treatment because of response (n=233; 33. 7%),

disease progression (n=54; 7. 8%), adverse events (n=31; 4. 5%), patients death (n=20; 2. 9%), other reasons (n=123; 17. 8%). **Conclusions.** Bortezomib, alone and combined with dexamethasone, is safe and effective in pretreated patients with relapsed/refractory MM in routine clinical practice. These results provide a useful comparator for data collected in the highly controlled clinical trial setting. Patients continue to be followed for long-term outcomes.

1508

RETROSPECTIVE ANALYSIS OF THROMBOCYTOPENIA IN RELAPSED MULTIPLE MYELOMA PATIENTS

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Background. Despite improvement in clinical outcomes in recent years, multiple myeloma (MM) remains incurable and most patients relapse after initial therapeutic response. Thrombocytopenia remains a clinical challenge in patients with relapsed/refractory disease. High-grade thrombocytopenia at our institution is primarily managed with platelet transfusion and chemotherapy dose modification. **Aims.** We hypothesize that thrombocytopenia is an important variable in the management of relapsed MM. As there are currently no studies in the myeloma literature that assess the impact of thrombocytopenia, we conducted a retrospective chart-review to evaluate the impact of thrombocytopenia in relapsed MM. **Methods.** Participants included all patients with relapsed/refractory MM who enrolled on clinical trials at Dana Farber Cancer Institute between January 1, 2007 and December 31, 2009. As some patients participated in multiple clinical trials during this period, therapy administered to a particular patient in a single clinical trial is termed a "treatment regimen. "We determined the overall incidence of thrombocytopenia (platelet count < 150,000/mcl) and the incidence of more severe thrombocytopenia (platelet count < 100,000/mcl or < 75,000/mcl) at treatment initiation. Medical record data collected to assess the clinical impact of thrombocytopenia included the following: episodes of ≥ grade 3 thrombocytopenia, number of platelet transfusions per month, treatment delays and discontinuations due to thrombocytopenia, occurrence of bleeding events, and overall survival from start of initial clinical trial within this period. Point estimates with exact binomial confidence intervals and median and range are used to describe the data. The differences in platelet transfusion requirements were assessed by the Wilcoxon rank sum test. **Results.** Data was abstracted for 125 patients on 202 treatment regimens. 71 patients previously underwent autologous stem cell transplantation. The overall incidence of thrombocytopenia (platelet count < 150,000/mcl) at time of treatment initiation was 45% (90 of 202 regimens). In 45 regimens (22%, 90% Confidence Interval (CI) 18%, 28%) platelet count at time of treatment initiation was < 100,000/mcl. The platelet count at time of treatment initiation was < 75,000/mcl in 25 regimens (12%, 90%CI 9%, 17%). For regimens with platelet count < 100,000/mcl at study entry, median platelet transfusion requirement over 4 weeks was 1. 51 units (range 0-20. 29); in regimens with platelet count ≥ 100,000/mcl, the median plt transfusion requirement was 0 units transfusions per 4 week period (range 0-2. 9) ($p < 0.0001$). Twenty-five bleeding events were observed during a treatment regimen in 16 subjects. Treatment was delayed for 30 additional subjects due to thrombocytopenia, including 2 subjects removed from studies due to thrombocytopenia. Patients who began any therapy with a platelet count < 100,000/mcl experienced poorer overall survival compared to patients who began therapy with a platelet count ≥ 100,000/mcl (log-rank test, p=0. 0013) (Figure 1). Overall survival at 1-year among 125 pts was 77% (90% CI [70%;83%]).

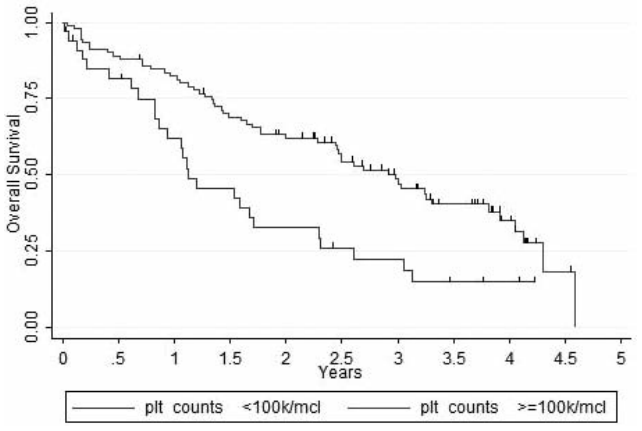

Figure 1.

Conclusions. Thrombocytopenia is a common problem in patients with relapsed/refractory MM, with poorer 1-year overall survival noted in patients with plt < 100,000/mcl at treatment initiation. Clinical studies assessing novel compounds to treat thrombocytopenia or minimize transfusion support are desperately needed in this patient population.

1509

FOLLOW UP OF MULTIPLE MYELOMA PATIENTS WITH RENAL IMPAIRMENT DURING A 10 YEAR PERIOD

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Background. Renal impairment (RI) is a relatively common feature of multiple myeloma (MM) and it has been shown in several studies that RI at diagnosis correlates to inferior survival, significant morbidity and increased early death rate. **Aims.** To compare the efficacy of bortezomib-based treatment to other anti-myeloma treatments during the course of the disease in patients presenting with creatinine ≥130 µmol/L. The primary endpoint of the study was renal response (RR) in 1st, 2nd and 3rd line treatment. Time to progression (TTP), time to next treatment (TTNT), overall survival (OS) and MM response were the secondary endpoints. **Methods.** All treated MM patients with a s-creatinine ≥130 µmol/L that were diagnosed in clinical practice between January 2000 and July 2010 at Karolinska Huddinge were selected. This s-creatinine limit was selected in an attempt to cover all MM patients with GFR <50 mL/min. A previous study from our centre analyzing a larger myeloma database also showed that patients with a s-creatinine ≥130 µmol/L had a worse median OS (less than 2 years) compared to those with s-creatinine <130 µmol/L (approximately 4. 7 years). The study population was divided into those receiving bortezomib (Bz) and those receiving other drug combinations (control group). **Results.** Of the 556 patients that were diagnosed and treated with MM between 1st of January 2000 and 1st of July 2010, 95 patients had an s-creatinine ≥130 µmol/L at diagnosis. Of the 95 patients, 52 also had RI in 2nd line and 25 in 3rd line treatment resulting in a total of 172 treatment occasions where anti-myeloma treatment was given to patients with renal RI. There was no significant difference regarding age, sex, hemoglobin, β2-microglobulin, calcium, or albumin between the Bz-group and the control group. In the Bz-group, in 1st line treatment, 11 of 12 patients (92%) improved their GFR compared to 57 of 83 (69%) in the control group (p=0. 049). In the 2nd and 3rd treatment line the overall RR was 19 and 43% in the Bz treated compared to 23 and 28% in the control group (p=0. 749, and p=0. 257). 4 of the 12 patients (33%) receiving Bz in 1st line reached complete renal response (CRrenal) vs. 38 of 83 patients (46%) in the control group. When analyzing all treatment lines together the MM response was better in the Bz-group with significantly higher overall response rate of 80%, compared to 41% (p=0. 026). Median TTP in 1st, 2nd and 3rd line in the Bz-group was 18, 6 and 10 months and in the control group 11, 10 and 8 months. Median survival time was 3. 4 years for the control group, whereas 62% of the Bz treated patients still were alive at median time of follow up. **Conclusions.** RI is still an important prognostic marker in MM. Bz-based regimens give a higher frequency of RR and MM response, thus indicating an advantage for bortezomib in comparison to other treatment regimes for MM patients with RI.

1510

BORTEZOMIB CAN OVERCOMES ISS AS PROGNOSIS FACTOR IN PATIENTS WITH MULTIPLE MYELOMA (MM) HAVING HIGH DOSE THERAPY (HDT)

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Background. The outcome for MM patients has changed since the introduction of Bortezomib. Despite therapeutic advances, HDT remains the treatment of choice for eligible patients. Standard prognosis systems (i. e., ISS and DS), cytogenetic or GEP have been mainly evaluated before new active drugs. Current data suggest that novel agents can overcome adverse prognostic factors, i.e., deletion 13q and t(4;14) at diagnosis. **Aims and Methods.** Using our patient's database, we analyzed standard prognostic factors and transplant modalities on PFS and OS for 306 consecutive MM patients undergoing HDT from 1997 to 2009. 83 patients received Bortezomib + Dexamethasone and 223, VAD as induction regimen. When patients underwent tandem HDT (n=84), we considered only the first HDT. According to the induction therapy, we eval-

uated the influence of age, sex, isotype, DS and ISS staging systems, status before and post HDT, number of lines of therapy, time of HDT from diagnosis, dose of re-infused CD34(+) cells, bone marrow plasma cell infiltration (BMPCP) and beta2-microglobulin (SB2M) at diagnosis on OS and PFS. Data processing was performed using SAS software packages 9. 2. PFS measured from the date of first PBSCT to the relapse (Kaplan-Meier product limit method), and OS, from diagnosis to death or last follow-up. The association of potential independent variables to PFS and OS was assessed by univariate analysis using Kaplan Meier method and Log Rank Test, then, by multivariate analysis through Cox proportional hazards model. **Results.** Median age was 61 years (34-73). Sex ratio M/F was 1. 17. Fifty-four percent were IgG, 29%, IgA, 17%, light-chain. Median number of CD34 cells is 5. 08 (1. 2-26) x106/Kg. Forty-five percent of patients had SB2M less than <3. 5mg/L. Forty-eight percent had BMPCP ≥ 30%. Forty-one percent of patients were ISS I, 29%ISS II and 30 ISS III. Clinical DS staging was shared in 7% of stage 1, 18% of stage 2 and 75 % of stage 3 at diagnosis. Eighty-one% of PBSCT were preceded with one line of treatment before transplant. Ninety percent of patients have undergone one HDT and 80 % have received only one line of induction treatment. Median PFS follow-up was 45 months for VAD induction regimen (VIR) and 10 months for Bortezomib induction regimen (BIR). In VIR group, Isotype, SB2M, BMCP, sex, and post-transplant response are significant for PFS in univariate model. In Cox's model, BMPCP, sex, isotype, and post-transplant response are significant. In contrast, after BIR, only post-transplant response remains a significant prognosis factor (p<0. 0001). Isotype (p=0. 41), SB2M (p=0. 62), BMCP (p=0. 4), are not significant. Median OS follow-up was 57 months for VIR and 21 months for BIR. In VIR group BMPCP, post-transplant response, isotype, SB2M, age at transplant, and ISS were the most consistent prognosis factors correlated with OS. In Cox's model only BMCP, post-transplant response, isotype and ISS are significant. In the BIR group, only post-transplant response is a significant factor correlated with OS (p=0. 05). ISS (p=0. 73) nor SDD (p=0. 89) are significant (Figure 1). **Conclusions.** Bortezomib modifies usual prognosis scoring system as ISS or SDD.

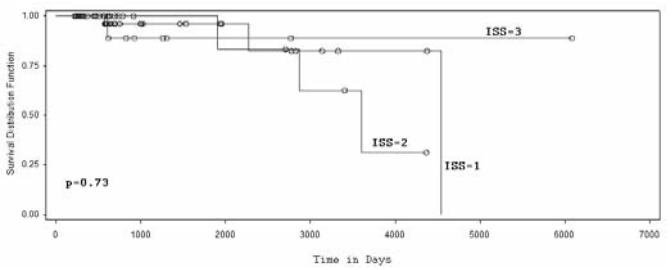

Figure 1. OS in Bortezomib group according to ISS.

1511

WITHDRAWN

1512

GOOD CLINICAL ACTIVITY AND FAVOURABLE TOXICITY PROFILE OF BORTEZOMIB, FOTEMUSTINE AND DEXAMETHASONE (B-MUD) FOR THE TREATMENT OF RELAPSED MULTIPLE MYELOMA PATIENTS

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Background. Fotemustine, a nitrosourea alkylating agent, has been shown effective as single agent in relapsed multiple mieloma (MM). The proteasome inhibitor Bortezomib represent a cornerstone for the treatment of MM. **Aims.** We explored the feasibility and the efficacy of the new combination bortezomib (B) + fotemustine (Mu) + dexamethasone (D) (B-MuD) in MM patients (pts) relapsed after at least one therapy. **Methods.** This phase II single-centre study has been approved by local ethical committee; all pts signed written informed consent. Experimental therapy consisted of escalating dose of Fotemustine (80-100 mg/m^2 i. v.) on day 1 associated to Bortezomib 1,3 mg/ m^2 i. v. on days 1,4,8,11 + Dexamethasone 20 mg orally on days 1-2, 4-5, 8-9, 11-12 of 21-day cycle for 6 cycles. The MTD of Fotemustine resulted to be 100 mg/m^2. Protocol was amended due to extra-hematological toxicity after the enrolment of the first five pts as follows: Fotemustine 100 mg/m^2 i. v. on day 1, once-weekly Bortezomib 1,3 mg/ m^2 i. v. on days 1, 8, 15, 22, Dexamethasone 20 mg i. v. on days 1, 8, 15, 22 for six 35-day cycles. **Results.** Twenty-four pts have been enrolled: M/F 13 (54%)/11(46%), median age 69 years (44-83), median number of previous therapies 2 (1-5). Previous treatments included autologous transplant in13 pts (54%), bortezomib in8 pts (33%), oral melphalan in11 pts (46%) and thalidomide in 15 (63%). Four pts discontinued for extra-hematological toxicity, 2 for progression. Twenty-two pts were evaluable for best response since completing at least 2 cycles. CR + VGPR was achieved in 11 patients (50%), 9 pts had PR (41%), 2 pts had SD (9%). Response rates on intention-to-treat-basis were as follows: CR 8%, VGPR 33%, PR 21%, SD 8%, Progression 4%. Median time to first response was 36 days (range 21-83) with a median DOR of 19. 4 months (95% CI 11. 6-23. 7 months). After a median follow-up of 24. 3 months (range 1. 6-32. 8 months), the median OS is 28. 5 months (95% CI 22. 1-NR), the median TTP and the median PFS are 20. 5 (95% CI 11. 9-22. 2 months) and 19. 1 (95% CI 11. 9-22. 2) months respectively. Median time to next therapy (TNT) was 16. 2 months (4-25. 2). As far as toxicity was concern, one-hundred and thirty-three AE of any grade were observed, 65 hematological (49%) and 68 (51%) non-hematological; among those there were 19 infections (14%), 31 neuropathies (23%), 18 gastrointestinal/metabolic events (14%). Thrombocytopenia was the most common AE. There were fifty-one (38%) grade 3-4 AE, most of which were haematological (66%). Eight SAE were observed: 5 infections, 2 neurological (1 disautonomic, 1 sensory-motor neuropathy), 1 gastrointestinal bleeding. Half of the events occurred during the first two cycles (49%), most were manageable, with 69% resolved or improved, 15% unchanged, 15% worsened. Need for dose reduction occurred in 65% of the events. **Conclusions.** Fotemustine in combination with dexamethasone and once-weekly bortezomib has a good clinical activity in relapsed MM pts, obtaining a high percentage of good quality and long-lasting response (50% of ≥VGPR, median DOR 19. 4 months). Safety data showed a good toxicity profile

1513

ASSOCIATION OF IL-17A AND IL-17F POLYMORPHISMS TO SUSCEPTIBILITY AND CLINICAL FEATURE OF MYELOMA IN JAPANESE PATIENTS

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Background. Multiple myeloma (MM) is characterized by unique clinical characteristics including anemia, bone lesions, and renal failure because of malignant plasma cell proliferation. The growth of plasma cell is dependent on a complex interplay among various cytokines, adhesion molecules and other factors in the tumor microenvironment. Dysregulation of several cytokines has been detected in some patients with MM, and was known to be associated with an adverse prognosis. Interleukin-17(IL-17) A and IL-17F are the members of the IL-17 cytokine family (IL-17A-F) responsible for the pathogenic activity of Th17 cells. Th17 was shown to play a pathogenic role in malignancy. Furthermore, it has been shown that IL-17 promotes myeloma cell growth in vitro and inhibits immune function in patients with MM. Polymorphisms of IL-17A (G-197A) and IL-17F (7488T/C) were studied in some diseases, including collagen disease and malignancy. However, there have been no previous reports of the influence of IL-17A and IL-17F polymorphism on the incidence and clinical outcome of MM. **Aims.** We examined the single nucleotide polymorphisms (SNPs) of IL-17A (G-197A) and IL-17F (7488T/C) in MM patients, and analyzed the relationship between IL-17A /IL-17F SNPs and the susceptibility and clinical features. **Patients and Methods.** We examined 93 patients [age range,35-83 years; stage Ⅰ(n=8), stage Ⅱ(n=23),stage Ⅲ(n=62); IgG (n=55), IgA (n=15),IgD (n=2),non-secretory(n=3), Bence Jones(n=18)] with MM and 150 healthy controls. Genomic DNA was isolated from peripheral blood using the DNA Kit (QIAGEN, Hilden, Germany). Genotyping was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Genotype and allele frequencies were compared between the study groups using χ^2-test. The characteristics and laboratory features of MM patients with each IL-17A and IL-17F polymorphisms were compared using χ^2-tests and student t-tests. The Kaplan-Meier method was used in the calculation of overall survival. Overall survival curves were compared with the log-rank test. Probability values <0. 05 were considered statistically significant. **Results.** IL-17A G-197A genotype and allele frequencies were not significantly different between MM patients and controls (compared with AA and non-AA genotype, GG and non-GG genotype). In MM patients, IL-17A non-GG genotype was significantly associated with advanced international staging system (ISS). The frequency of ISS stage≧Ⅱ was significantly higher in non-GG genotype (non-GG vs GG genotype: 79. 1% vs 57. 1%,p<0. 05). The patients with non-GG genotype had significantly shorter survival from diagnosis than the patients with GG genotype (median of survival from diagnosis, non-GG vs GG genotype, 4. 4years vs 6. 3 years, p<0. 05). There were no significant difference in genotype and allele frequencies between MM patients and controls.

In the clinical characteristics, there was also no difference between patients with TT and TC genotype. **Conclusions.** These results suggested that the IL-17A (rs2275913, G-197A) polymorphism may be associated with the susceptibility and clinical feature of MM in Japanese patients.

1514

PROGNOSTIC CONTRIBUTION OF UNINVOLVED SERUM FREE LIGHT CHAINS (USFLC) IN MULTIPLE MYELOMA (MM)

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Background. The prognostic value of serum FLC and their ratio FLCR in MM is now established. Although it is accepted that increased prognostic information provided by FLCR as compared to FLC is due to the fact that it reflects both monoclonal FLC serum levels and eventual suppression of the polyclonal secretion, the eventual prognostic contribution of usFLC per se has not been extensively studied. **Aims.** To study the possible prognostic contribution of usFLC with regard to time to first treatment (TFT) and overall survival (OS) in MM patients. **Patients and Methods.** 180 MM patients with available baseline serum FLC at diagnosis were studied. 25% of patients were in ISS stage 1, 25% in stage 2 and 50% in stage 3. MM type was IgG, IgA, light chain and IgD in 64, 22, 12 and 2% respectively. 116 patients were kappa-LC and 64 lambda-LC restricted. Serum FLCs were measured by nephelometry (FREELITE, The Binding Site Ltd. Birmingham, UK). FLCs values were considered depressed for any usFLC-kappa value below 0. 13 mg/L and any usFLC-kappa below 1. 04 that were the lowest normal range in our healthy control's group. Serum usFLCs were then assessed for time to first treatment (TFT) and overall survival (OS) by the Kaplan-Meyer method. Survival curves were compared by the log-rank test. **Results.** 25% of patients had depressed usFLCs. In patients with depressed usFLCs a reduced TFT was observed compared to the others (p<0. 0001), in addition their median OS was 32 months (range 1-63) versus 86 months (range 61-110) in patients with normal usFLCs (p=0. 01). **Conclusions.** Depressed usFLCs were shown to be an adverse prognostic factor in MM. Suppression of polyclonal immunoglobulins was part of Durie's MM minor diagnostic criteria; depressed usFLCs express the same. Their negative prognostic impact is very interesting given that it cannot be attributed to malignant plasma cells genetic defects but rather reflects a bone marrow microenvironment process related to the complex biology of this disease.

1515

HEMATOLOGIC SAFETY PROFILE OF SINGLE-AGENT CARFILZOMIB IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA FROM FOUR PHASE 2 STUDIES

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Background. Carfilzomib is a next-generation proteasome inhibitor that is currently being evaluated in patients with multiple myeloma (MM). The majority of patients with MM exhibit either disease- or therapy-related myelosuppression sometime in the course of their disease. Therefore, detailed evaluation of treatment-emergent hematologic safety data with carfilzomib is of significant interest. **Aims.** To report the hematologic safety profile for single-agent carfilzomib from 526 patients with relapsed and/or refractory (R/R) MM treated in four phase 2 studies. **Methods.** Patients treated with carfilzomib in trials PX-171-003-A0, PX-171-003-A1, PX-171-004, and PX-171-005 were included in the analysis. Patients with preexisting hematologic abnormalities of Grade 0-2 (Grade 0"'3 for 005) were eligible to enroll. Carfilzomib was dosed in all studies on Days 1, 2, 8, 9, 15, and 16 of a 28-day cycle (C). Doses were 20 mg/m^2 in C1 for all studies, escalating to 27 mg/m^2 in C2 as defined per individual protocol, except 005 (15 mg/m^2 in C1, 20 mg/m^2 in C2, and 27 mg/m^2 in C3). Thrombocytopenia, lymphopenia, neutropenia, and anemia were adverse events (AEs) of interest, and data on incidence, frequency, and severity were analyzed. Laboratory data were analyzed for trends over time. **Results.** Summary of hematologic events*Defined as death or immediate risk of death; AE resulting in inpatient or prolonged hospitalization, surgery, or disability (or could lead to without intervention); or congenital birth defect of offspring. In general, platelet counts trended down, reached a nadir by Day 8 of a 28-day treatment cycle, and normalized before the start of the next cycle. There was no evidence of cumulative thrombocytopenia or clinically significant episodes of bleeding associated with thrombocytopenia. Febrile neutropenia was reported in 1% of patients. No mortality due to hematologic AEs was reported. **Conclusions.** Hematologic AEs, although common with carfilzomib treatment, were infrequently dose limiting. Clinically significant Grade 3/4 cytopenias were both infrequent and transient. The hematologic safety profile of carfilzomib was similar to or better than currently approved MM therapies, providing further evidence of its acceptable safety profile in heavily pretreated MM patients and indicating its potential widespread use in this patient population.

1516

PLASMA CELL LEUKEMIA: SUSTAINED RESPONSES ARE POSSIBLE WITH INNOVATIVE TREATMENT STRATEGIES

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Background. Despite markedly improved survival rates for MM, prognosis in a highly aggressive subset - plasma cell leukemia (PCL) remains dismal. Patients (pts) with primary PCL (pPCL) and with secondary PCL (sPCL) have a median overall survival (OS) of 11. 2 months (mo) and 1. 3 mo, respectively from the time of diagnosis (Tiedeman RE, 2008). CIBMTR data was encouraging in pts with pPCL showing progression free survival (PFS) of 34% and OS of 64% at 3 years (Mahindra A, 2011). Recently, GIMEMA group demonstrated that bortezomib-containing combinations as frontline therapy resulted in 55% OS and 42% PFS at 2 years (Arena GD, 2011). **Aims.** We evaluated our institutional experience in this high-risk subset of pts with intent to improvise outcomes with prolonged treatment strategies. **Methods.** We identified 28 pts (22 with pPCL and 6 with sPCL) among 1400 pts with plasma cell dyscrasias from our myeloma database from 01/2005 till 02/2012. Additional high risk features present include [(del17p -18&33%, t(4;14)-5&0%, t(14;16)-14&0% by fluorescence in situ hybridization or metaphase cytogenetics; hypodiploidy-32&33%,del13-40&50% for pPCL & sPCL pts, respectively. The endpoints of interest are PFS, OS, and response rates after ASCT and during maintenance. **Results.** In the pPCL subset, 20 of the 22 pts underwent initial bortezomib-based cytoreduction therapy (VTD-PACE in 12 pts; RVD/VTD in 13 pts). 21 pts underwent ASCT at a median of 6 months from time of diagnosis. Maintenance therapy post-ASCT was started in 12 pts with RVD (RVDm); 1 pt with bortezomib and 5 pts with lenalidomide. Overall median PFS is 66% at 3 years and OS is 73% at 4 years. Median PFS for pts on RVDm vs. no RVDm was 38 mo vs. 15 mo; p=0. 004. OS for pts on RVDm vs. no RVDm was 100% vs. 67%; p=0. 018. Achieving ≥PR vs. < VGPR post-ASCT (PFS: 38 vs. 14 mo; p=0. 002 and OS: 49 vs. 26 mo; p=0. 037) were significant for prolonged PFS and OS. Response rates for pPCL that underwent ASCT and maintenance are summarized in the Table 1. In the sPCL subset, median PFS is 3 months (0-9. 86) and OS is 3 months (0-11. 42). Duration from initial diagnosis of MM to diagnosis of sPCL >30 months vs. <30 months (median PFS: 1 mo vs. 8 mo and OS: 2 mo vs. 9 mo), presence of deletion 17p13 vs. none (median PFS: 1 mo vs. 8 mo and OS: 9 mo vs. 1 mo) are associated with worse outcomes. **Conclusions.** Cytoreduction with bortezomib-based regimens followed by early ASCT and continuing RVD maintenance delivers superior and sustained response rates and prevents early relapses in pPCL. Outcome data for sPCL are still disappointing and newer approaches should be considered. Safety data and tolerability of RVDm will be presented.

Table 1. Response rates for pPCL that underwent ASCT and maintenance.

	Pre-ASCT response (n=21)		Post-ASCT response (n=20)		Best response with maintenance (n=20)	
	n	%	n	%	n	%
sCR	2	10	5	25	6	30
CR	4	20	4	20	4	20
sCR + CR	6	30	9	45	10	50
VGPR	3	15	8	40	8	40
≥VGPR	9	45	17	85	18	90
PR	10	50	1	5	1	5
≥PR	19	95	18	90	19	95
SD	0	0	0	0	1	5
PD	2	10	1	5	0	0

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ADVANCES IN THE TREATMENT OF MULTIPLE MYELOMA: SURVIVAL ANALYSIS OF 560 PATIENTS IN A TWENTY-FIVE YEARS PERIOD IN A SINGLE CENTER

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Background. Therapy of multiple myeloma (MM) has been greatly advanced by introduction of autologous hematopoietic stem cell transplantation (AHSCT) in 90's, and appearance of new potent drugs thalidomide, bortezomib and lenalidomide in the last decade. **Aims.** to assess the efficacy of new treatment modalities on survival of MM patients treated at the University Hospital Center, Zagreb. PATIENTS AND **Methods.** From 1985 till 2010, 560 consecutive MM patients (pts) were analyzed. Median age at diagnosis was 60 (range 28-89) years. Pts were divided into three groups according to time period of available treatment modalities: Group one treated from 1985-1995, period prior AHSCT (n=158, median age 63 [range: 31-87]); group two treated from 1996-2001, period with AHSCT (n=139, median age 58 [range: 29-87]); and group three treated from 2002-2010, period with AHSCT+bortezomib+thalidomide (n=263, median age 60 [range: 28-89]). Each group was subdivided in two subgroups according to age at diagnosis indicating eligibility for AHSCT (<65 and ≥65 years of age). Estimated median overall survival (OS) was calculated by Kaplan-Meier method. Differences between groups were tested by 2-tailed log-rank test with p value of <. 05 being statistically significant. **Results.** Median follow-up for entire cohort was 42. 5 (range: 1-267 months). Seventy-three pts were lost from follow-up after median time of 16 (range: 1-123) months. Estimated median OS was 69 (95%CI 59. 07-78. 93) months. For groups 1 and 2, estimated median OS were 38 (95%CI 29. 44-46. 56) and 47 (95%CI 36. 06-57. 94) months respectively, while for the group 3 estimated median OS was not reached for the median follow-up of 48 (range: 1-118) months. OS was significantly different between all groups: groups 1:2 (p=0. 009), groups 2:3 (p<0. 0001), groups 1:3 (p<0. 0001) (Figure 1). When adjusted for age, significance between groups 1 and 2 was not reached (p=0. 058) although showing trends for better OS in group 2. Statistically significant difference remained between groups 1:3 and 2:3 (p<0. 0001, for each). OS for patients younger than 65 are significantly better compared to patients with 65 years or older, in all three groups. **Conclusions.** The analysis of OS clearly showed significantly better outcome for pts treated with AHSCT, especially for younger patients. In most recent time period both younger and older patients showed better OS probably due to introduction of new treatment modalities with thalidomide or bortezomib. Results of the study are in accordance with other similar retrospective analyses.

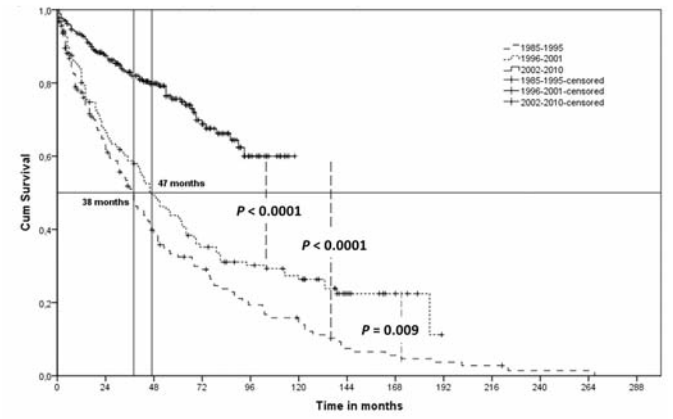

Figure 1. Overall survival for multiple myeloma patients in different periods.

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LENALIDOMIDE COMBINED WITH LIPOSOMAL DOXORUBICIN AND LOW DOSE DEXAMETHASONE (RDD) FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: SAFETY AND EFFICACY

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Background. Multiple Myeloma treatment has considerably changed in the past decade with improvement of response rates and overall survival. Nevertheless, the best treatment regimen for relapsed patients is not standardized. Lenalidomide is approved for treatment of relapsed myeloma. Previous trials have highlighted activity of Liposomal Doxorubicin in this setting of patients. **Aims.** We asses safety and efficacy of Lenalidomide,Liposomal Doxorubicin and low dose dexamethasone (RDd) in relapsed/refractory patients. **Methods.** between June 2008 to February 2012,35 patients were enrolled. Lenalidomide(25 mg/die,day 1-21), Liposomal Doxorubicin (30mg/mq i. v. on day 1) and dexamethazone (20 mg/die, day 1, 8, 15, 22) of 28 days cycle, was administered for 6 cycles (Induction Phase,IP). Patients in CR-PR(IMWG criteria) post IP received 3 other courses of RDd as consolidation. Responder patients allowed 10 mg/die Lenalidomide (day 1-21 every 35 days) until progression or treatment intolerance. Unresponsive patients discontinued treatment. **Results.** The characteristics of enrolled patients were summarized in Table 1.

Table 1. Patient characteristics at enrollment.

Patient Characteristics	N=35
Median age, y (range)	69 (46-81)
⩾ 65 years	21(60%)
Type of Myeloma, no. (%)	
IgGL/IgGK	8 (23%)/ 12 (34%)
IgAL/ IgAK	4 (11%) / 8 (23%)
Lambda	2 (6%)
Non secer	1 (3%)
Durie Salmon stage, no. (%)	
IIIA	27 (77%)
IIA	6 (17%)
IIIB	2 (6%)
ISS, no. (%)	
I	12 (36%)
II	13 (40%)
III	8 (24%)
Median Time since initial diagnosis, months	31 (5-185)
Median number of prior therapy, no.(range)	2 (1-6)
cycles>3	9 (26%)
Prior transplant, no. (%)	17(49%)
Prior thalidomide regimens, n (%)	19 (54%)
Prior Bortezomib regimens, n (%)	24 (69%)

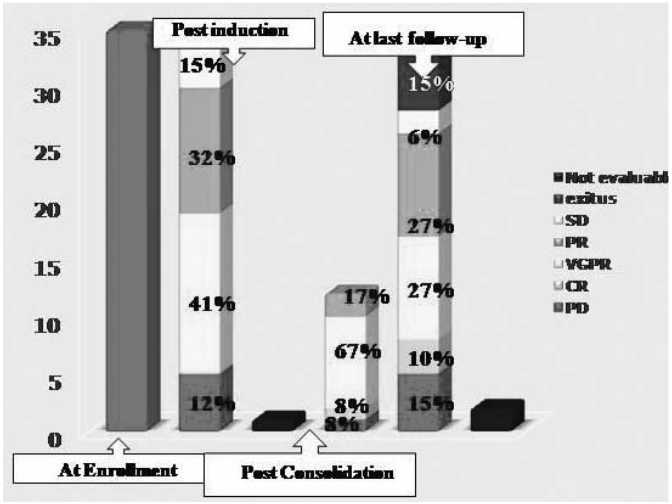
Nine patients (26%) were considered as high risk because of heavly pre-treatment. Nineteen (54%) and 24(69%) received,respectively,previous treatment with thalidomide or bortezomib,Seventeen patients(49%) received a previous autotransplant. The common side-effects during RDd were showed in Table 2.

Table 2. Incidence of haematological and extra-haematological toxicity.

Variable	All cases 35	WHO grade, n (%)			
		1	2	3	4
Haematological side effects					
Anemia	23 (66%)	15 (65%)	5 (22%)	3 (14%)	/
Thrombocitopenia	22 (63%)	14 (64%)	3 (14%)	5 (27%)	/
Neutropenia	33 (94%)	15 (45%)	10 (30%)	8 (26%)	/
Non-Haematological side effects					
Neuropathy	24 (69%)	19 (79%)	5 (21%)	/	/
Skin rash	4 (12%)	3 (75%)	1 (25%)	/	/
Fever	6(17%)	1	1		
Deep Venous Thrombosis	4(11%)				

No grade4 haematologycal toxicity was observed. The G-CSF support was required in 33 patients (94%). GradeI-III neutropenia was documented in 33 patients,however febrile neutropenia occurred in 6 patients (17%). The major extra-haematological adverse event was neuropathy occurred in 24 patients (69%). Deep venous thrombosis occurred in 4 patients (11%). Lenalidomide dose reduction or temporary discontinuation occurred in 8 (23%) and 17 (49%) patients, respectively. Patients aged ³65 years showed higher incidence of haematological toxicity (anemia 78%, p=0. 004, requiring erythropoietin; thrombocytopenia 77%, p=0. 01 and neutropenia requiring G-CSF support (100%,p=0. 08). Elderly patients delayed administration of RDd more frequently (77%vs 23%, p=0. 05). After IP, the overall response rate (ORR:11PR+14VGPR) was 73% (Figure 1). Four patients (12%) were in Stable Disease (SD). Four of responder patients relapsed (14%). Twelve patients completed the planned treatment. After consolidation, the ORR was 92%: 1CR (8%),8 VGPR (67%) and 2 PR(17%). No differences in terms of ORR were observed in elderly patients (p=0,8). Six of seven patients (86%) younger than 65years and refractory to first line therapy obtained at least a PR and were transplanted or are waiting for transplant. After a median follow-up of 15 months (2-37),5 patients(14%) died(3 for disease progression,1 for infection and 2 for transplant-TRM). Twenty-eight patients are alive:3 (11%) in CR,9 (32%) in VGPR,9(32%) in PR,2 (6%)in SD and 5(19%) in progression disease. The median TTP is 9months (3-25). Kaplan Meyer estimated 1,5yTTP is 52%. We analyzed the factors influenced TTP: only previous treatment with thalidomide seem to be correlated to a lower TTP (p<0,004). **Conclusions.** Our experience suggests that RDd is both tolerable and effective for second line Multiple Myeloma therapy. The response rate and quality of response considerably improve during the different phases of planned treatment. This combination should be considered appropriate treatment in patients with high risk refractory/relapsed Multiple Myeloma, who are aged>65 and/or received >2prior line therapies (including thalidomide,bortezomib and autotransplant). RDd is also an optimal therapy in refractory younger patients as bridge to transplant.

Figure 1. Response rates during the different phases of treatment.



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SUBCUTANEOUS VERSUS INTRAVENOUS ADMINISTRATION OF BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA: EXPERIENCE IN A SINGLE INSTITUTION

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Background. Bortezomib (VELCADE) is approved in the US and Europe for the treatment of patients with multiple myeloma (MM) in both the frontline and the relapse settings. The recommended dose of bortezomib is 1. 3 mg/m2 administered as a 3- to 5-second bolus intravenous (IV) injection. As an alternative to IV delivery, subcutaneous (SC) administration of bortezomib may be a good option. **Methods.** 55 patients diagnosed of MM were treated between January 2007 until February 2012 with SC (24 patients) or IV (31 patients) bortezomib (1. 3 mg/m2 on days 1, 4, 8 and 11 every 21 days for a total of 6-8 cycles or 1. 3 3 mg/m2 on days 1, 4, 8, 11, 22, 25, 29 and 32 for 1 cycles and on days 1, 8, 22 and 29 for 8 cycles). The median of treatment cycles were 6 (2-13). One patient received Thalidomide (50 mg/day) after first cycle and another one after 6th cycle. SC injection sites were the thighs or abdomen, and the injection site was rotated for subsequent injections within a cycle. IV injections were administered at a concentration of 1 mg/ml as a 3- to 5-second IV push, and SC injections were administered at 2. 5 mg/ml. The primary endpoint was overall response rate (ORR; complete response [CR + PR]) after 6-8 cycles. Safety and tolerability of the two administration routes, including local tolerability of SC administration, were also assessed. **Results.** Because of treatment-emergent peripheral neuropathy it was necessary reducing the doses in 33% vs 41% of the patients in the sc and iv group respectively Thrombocytopenia (Grade 3 and higher) were reported in 4 of 24 (17%%) patients in the subcutaneous group and 13 of 31 (42%) in the intravenous group. 75 % of the patients were able to complete treatment. Overall response rate were similar in both groups. SC administration of VELCADE also demonstrated local tolerability. **Conclusions.** Rates of peripheral sensory neuropathy were lower with subcutaneous than with intravenous bortezomib, as were rates of diarrhoea and thrombocytopenia. None case of fatigue or hypotension were reported in the sc group. Subcutaneous administration of bortezomib is non inferior to the standard intravenous route in patients with multiple myeloma and seems tohave an improved systemic safety profile.

1520

LENALIDOMIDE IN POEMS SYNDROME

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Background. POEMS syndrome is a rare multisystemic disease. Vascular endothelial growth factor (VEGF) correlated with the activity of the disease and it could account for clinical manifestations. There are no controlled trials and no established treatment. Lenalidomide. has anti-angiogenic activity through inhibition of VEGF and TNF alpha. **Aims.** his study plans to evaluate efficacy of Lenalidomide in POEMS SYNDROME. **Patient sand Method.** From 10/09, we started a pilot study with Lenalidomide plus Dexamethasone(RD) in pts pretreated or with newly diagnosed POEMS syndrome. Lenalidomide 25 mg/day was given for 21 days in association with weekly Dexa 40 mg until tolerated or progression. Primary objective of this study was evaluation of clinical efficacy of RD combination in terms of improvement of either the disability caused by the neuropathy (ONLS) or organ specific symptoms after 6 cycles. Secondary objectives were improvement of muscle strength assessed by the expanded MRC sum score and improvement of sensation assessed by the INCAT sensory sum score. After first 6 cycle, pts were evaluated for response. At this time, 13 pts have been enrolled, (11 men and 2 woman), with median age of 51 yrs (range 45-76). All pts were pretreated and 2 had received transplant; in all pts was detected monoclonal component (MC) (IgAL in3 pts,IgGL in9 pts,cL light chain in 1) and sensory/ motor peripheral neuropathy. Sclerotic bone lesions was evident in6 pts, endocrinopathies in 9, skin changes in 9, peripheral edema in 10, organomegaly in 8, lymphoadenophaty in 4, papilledema in 7, hrombocytosis in4 pts. VEGF serum level was elevated in all patient with a median value of 3428 pg/ml (range 1400-8978). **Results.** 9 pts are still on treatment and received a median of 16 RD cycles (range 2-35). 8 pts are evaluable for response after 6 cycles. A clinical response, with improvement of all clinical manifestationsof disease, was observed in all pts. Neurological improvement, was evident already after 3 RD cycles, confirmed by nerve conduction studies after every 6 cycles. One patient with tetraparesis actually is able to walk without devices, and his upper limb strength is normal with improved sensory disturbances. Only in 3 patients MC disappeared. VEGF decreased in all treated pts: median 3428 pg/ml (range 1400-8978) before treatment to 1500 pg/ml (467-3579). 3 pts discontinued treatment:1 withdraw consent, 1 drop-out after 4 cycles wit progressive disease and 1 died for pulmonary infection on cycle 1. Lenalidomide reductions was necessary in3 pts (1 extraematological toxicity and 2 thrombocytopenia) and dexa in7 pts. **Conclusions.** Lenalidomide is efficacy and well tolerated in POEMS syndrome, with rapidly and continuous neurological improvements. Correlation between response and VEGF level was confirmed. At this time no responding pts experienced disease progression. The studyaccrual is still ongoing.

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SERUM FREE LIGHT CHAIN (FLC) AND HEAVY/LIGHT CHAIN (HLC) ASSAYS PROVIDE SENSITIVE AND QUANTITATIVE METHODS FOR MONITORING IGA MULTIPLE MYELOMA PATIENTS

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Background. Serum protein electrophoresis (SPE) is the standard method for monitoring monoclonal immunoglobulin (M-Ig) concentrations in multiple myeloma (MM) patients. However, the utility of the test may be limited in patients with low M-Ig load, light chain only disease or when the M-Ig co-migrates with other serum proteins, typically in IgA MM patients. Furthermore, clonal evolution characterised by subtle changes in monoclonal protein expression may not be identifiable using standard electrophoretic techniques. **Aims.** To assess the utility of FLC and IgA HLC immunoassays as tools to monitor IgA MM patients. **Methods.** FLC and HLC were measured nephelometrically in retrospective serial samples from 30 IgA (20 IgAκ; 10 IgAλ) MM patients; results were compared with historic clinical evaluations. Survival analysis at maximum response was performed using SPSSv19. **Results.** At presentation, FLC ratios were abnormal in 29/30 and HLC ratios were abnormal in all 30 IgA MM patients, including 9 patients with non-quantifiable SPE. 27/30 patients responded to therapy (12 PR, 6 VGPR, 9 CR). The FLC ratio was abnormal in all patients achieving a PR and in 5/6 patients achieving a VGPR. The HLC ratio remained abnormal in all patients achieving PR or VGPR. In patients achieving CR, FLC and HLC ratios were normal in 5/9 and 4/9 patients, respectively. The 5 patients with an abnormal HLC ratio at CR had a shorter overall survival compared with 4 patients with a normal HLC ratio (median OS: 981 days v not reached; p=0. 019). During follow-up, FLC and HLC ratios were abnormal in 11/30 (37%) patients with non-quantifiable SPE. Furthermore, monitoring with HLC and FLC identified clonal changes in 3/30 (10%) patients. An IgAκ patient presented with 29g/L IgAκ and 92mg/L FLCκ, responded to therapy and achieved a VGPR after 243 days. At this time both HLC and FLC ratios decreased but remained abnormal. The patient subsequently relapsed after 713 days; HLC and FLC ratios both increased at this point. The patient responded to a second line of treatment and a secondary response was recorded after 798 days; HLC and FLC ratios reduced in line with this response but remained abnormal. The patients terminal relapse was characterised by an increasingly abnormal HLC ratio, however FLC production did not increase, suggesting a subtle change in M-Ig production. A second IgAk patient presented with 48g/L IgAκ and 400mg/L FLCκ. The patient achieved CR after 492 days which was characterised by normalisation of both HLC and FLC ratios. The patient relapsed after 1122 days which was characterised by IgAκ production only, with normal FLCκ production and FLC ratio. The patient responded to second line therapy; however HLC ratios did not normalise. The patients terminal relapse was characterised by both M-Ig and FLCk production. Clonal changes were identified in one further patient who had FLC escape 822 days following response. **Conclusions.** HLC provides a sensitive method for identifying and quantifying M-Igs that are difficult to detect by SPE. Furthermore, monitoring patients with HLC and FLC can detect clonal evolution which may be missed using standard techniques.

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ASSESSMENT OF IGA HEAVY/LIGHT CHAIN IMMUNOASSAY UTILITY IN MULTIPLE MYELOMA PATIENTS

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Background. Current international guidelines recommend serum protein electrophoresis (SPEP) and serum free light chain (FLC) for the identification of monoclonal immunoglobulins (M-Ig), reflexed to immunofixation (IFE) for confirmation and typing. In the case of intact immunoglobulin monoclonal gammopathy of undetermined significance (MGUS) or multiple myeloma (MM) SPEP is also recommended to monitor the protein-Ig. However, co migration of the M-Ig with other serum proteins can make SPEP inaccurate, a common occurrence with IgA and IgM and less so with IgG M-Ig's. In these instances total immunoglobulin measurements are recommended. However, total immunoglobulin may be of insensitive as the patients respond to therapy or in cases of low M-Ig production it cannot distinguish between monoclonal and polyclonal immunoglobulins. Newly developed heavy/light chain (HLC) assays can be used to quantify IgAκ and IgAλ, enabling calculation of an IgAk/IgAl ratio. Here, we assess the utility of IgA HLC in the identification and monitoring of IgA monoclonal immunoglobulins, including those with difficult to quantify SPEP results. **Methods.** IgA HLC analysis was performed retrospectively on samples from 53 IgA (22 IgAk; 31 IgAl) MM patients at various stages of treatment attending the Haematology Dept atSt HelierHospital. The HLC IgAk/IgAl normal range was 0. 80-2. 04. **Results.** 10/53 (19%) samples (8 IgAk / 2 IgAl) had quantifiable IgA by SPE (median 5g/L, range 1-40g/L); all 10 samples had abnormal HLC ratios. Due to inaccuracies in SPEP measurement (such as co-migration with other proteins) in the remaining 43 samples (23 IgAk / 20 IgAk), total IgA measurements were used to quantify the immunoglobulin (median 5g/L, range 1-58g/L). In 14/43 samples total IgA levels were less than 5g/L; 8 of these samples had an abnormal IgA HLC ratio; the remaining 6 samples had a normal ratio, however no IFE data was available to determine if these patients had a persistent monoclonal protein. Sequential monitoring samples (median n= 5, range 2-9) were available for 23 patients, in 2/23 patients SPEP measurements were reliable and in each patient HLC accurately monitored patient responses. Of the remaining 21 patients whose monoclonal protein was measured using total IgA, there were similar results between HLC and total immunoglobulins in 11 patients during the period of assessment. In 10/21 patient total IgA was below 5g/L, in all cases abnormal HLC ratios indicated the presence of a monoclonal protein. Furthermore, in 3 patients HLC ratios provided additional information, in 2 patients an increasingly abnormal HLC ratio indicated relapse before the level of monoclonal protein exceed 5g/L. This relapse was identified due to sequential suppression of the uninvolved polyclonal IgA. In 1 patient originally characterised as a FLC MM patient an intact immunoglobulin producing clone was identified following chemotherapy. **Conclusions.** IgA HLC ratios are able to sensitively detect clonal production even when SPE is normal or when total IgA is within the normal range.

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IMPROVED SURVIVAL IN MULTIPLE MYELOMA: A POPULATION-BASED STUDY SINCE 1985

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Background. A great effort has been made to improve overall survival (OS) in patients with multiple myeloma (MM). However, MM remains an incurable disease and progress in elderly people is still poor. **Aims.** To assess the OS pattern in MM using population-based data from The Granada Cancer Registry (GCR), during the last 27 years. **Methods.** All patients diagnosed with MM from 1985 to 2012 were registered in the GCR and included for OS analysis. Patients were initially assigned to three groups according to time of diagnosis: 1985-1994, 1995-2004 and 2005-2012. Clinical data were available from 1993 and we therefore divided patients in two groups: 1993 to 2002 and 2003 to 2012. On the basis of the age limit of 65 years, we constructed survival curves according to Kaplan-Meier method. Log-rank test was used to compare OS curves. The statistical package used was R. **Results.** 490 patients have been diagnosed with MM during the period of study, 226 men and 262 women (53,69 %). Median age was 67 years (21-91). Median OS was 25 months (19. 6-30. 4). There was a significant trend of increasing OS in the three groups (p=0. 0072). Patients diagnosed during the last decade (2003-2012) (n=174) had a better survival than patients diagnosed from 1993 to 2002 (n=207): median OS increased from 21 to 34 months (p=0. 002). This effect depends on the age group: for patients younger than 65, median OS improved from 25 to 49 months (p=0. 013); however, for those older than 65, the impact on median OS is 18 to 31 months (p=0. 130). We confirm the prognostic value of the International Staging System (p= 0. 0108). We found a significantly shorter OS for patients with a positive hepatitis C virus serology (p= 0. 0123). The body mass index (p= 0. 00266) and the M-protein type (p= 0. 0579) also might have a prognostic role. Conventional karyotyping is pathological only in 22%. FISH analysis is not routinely made until recently and is not available for comparison. **Conclusions.** MM is a heterogeneous disease with survival times ranging from a few days to more than ten years. Treatment strategy depends largely on age. Outside clinical trials, OS in real life patients with MM is still disappointing. Encouraging results have been shown with new agents combinations, but the impact in OS in the elderly needs to improve

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ELOTUZUMAB-ASSOCIATED INFUSION REACTIONS: INCIDENCE AND MANAGEMENT

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Background. Elotuzumab is a humanized monoclonal antibody (mAb) targeting CS1, a cell surface glycoprotein highly expressed on >95% of multiple myeloma (MM) cells. In a phase 1/2 study 1703, in patients with relapsed/refractory (RR) MM, elotuzumab plus lenalidomide and low-dose dexamethasone demonstrated an objective response rate of 82% in both phase 1 (n=28) (1) and in the combined treatment group of ongoing phase 2 (n=73) (2). Median progression-free survival has not been reached yet in the phase 2 study, after a median follow-up of 14. 1 months (2). Therapeutic proteins, including mAbs, can induce infusion reactions (IRs) associated with flu-like symptoms, which can be mitigated by premedication. Previous reporting of IRs after elotuzumab infusion was based on pre-defined adverse events (AEs) that could potentially be IRs and did not include investigator assessment of relationship to elotuzumab or reporting of IRs that led to infusion interruption (1,2). **Aims.** To report investigator-designated IRs (IDIRs), IRs resulting in infusion adjustment, and the impact of a premedication regimen on incidence and severity of IRs in the phase 1/2 study 1703. **Methods.** Patients (N=101) with RR MM received 5, 10, or 20 mg/kg of elotuzumab IV (days 1, 8, 15, and 22 every 28 days in first 2 cycles and days 1 and 15 of subsequent cycles), lenalidomide 25 mg PO (days 1-21) and dexamethasone 40 mg PO weekly. Thirteen phase 1 patients (n=28) received no premedication prior to elotuzumab infusion; 15 patients received partial premedication and were excluded from the analysis. All patients in the ongoing phase 2 study (n=73) received a premedication regimen of corticosteroids (IV methylprednisolone 50 mg or IV dexamethasone 8 mg), H1/H2 blockers, and acetaminophen prior to elotuzumab infusion. IDIRs were identified by the investigator as a sign/symptom of an elotuzumab-related IR, and independently as IRs requiring elotuzumab infusion adjustment - interruption, rate decrease, or discontinuation. **Results.** Table 1 summarizes the incidence and severity of IRs resulting in elotuzumab infusion adjustment in patients with and without premedication. Two patients not treated with premedication experienced grade 3/4 IRs and withdrew from treatment; no patient receiving premedication withdrew from treatment due to IR. After incorporation of premedication, grade 3 and all grade IDIRs were reported in 1/73 (rash, 1. 4%) and 9/73 (12. 3%) patients, respectively. No grade 4/5 AEs were reported. The most common IDIRs were nausea and pyrexia (4. 1% and 2. 7% of patients, respectively). **Summary and Conclusions.** Study protocol-guided, steroid-based premedication decreased the incidence and severity of elotuzumab-related IRs requiring elotuzumab infusion adjustment, and did not appear to affect clinical efficacy of the treatment. Assessment of IDIRs is a clinically relevant method of capturing elotuzumab-induced IRs. Analysis of IDIRs will continue in the current and ongoing randomized phase 3 studies (ELOQUENT-1; CA204-006; NCT01335399 and ELOQUENT-2; CA204-004; NCT01239797). 1. Lonial S, et al. *J Clin Oncol* 2012 (in press). 2. Lonial S, et al. *Blood* 2011; 118: Abstract 303.

Table 1.

	No premedication (n=13)		Premedication (n=73)	
	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)
IR resulting in infusion adjustment	3 (23.1)	2 (15.4)	3 (4.1)	2 (2.7)

1525

COMPARISON OF EFFICACY OF IMMUNOGLOBULIN FREE LIGHT CHAIN (FLC), HEAVY CHAIN/LIGHT CHAIN (HLC) ASSAYS AND IMMUNOFIXATION (IFE) IN ASSESSMENT OF REMISSION IN MULTIPLE MYELOMA (MM)

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HLC separately measures in pairs the light chain types of each intact immunoglobulin (Ig) class generating ratios of monoclonal Ig/ non-involved polyclonal Ig concentrations; potentially improving assessment of monoclonal protein response. Normalization of FLC ratio is considered a higher level of complete remission (CR) in MM and has been incorporated into definition of stringent CR (sCR) in IMWG Uniform Response Criteria. Recently, there was reported that abnormal HLC after induction therapy has a high negative predictive value for identifying patients not achieving CR by uniform response criteria and is also associated with shorter progression free survival (PFS) after transplant. FLC ratio normalization had no impact suggesting that stringent CR criteria may need further validation (Hari et al. Blood 2011; 118: 1241). To compare IFE, HLC and FLC results in assessment of remission and to assess whether CR with using the FLC ratio and HLC ratio criteria is prognostic for PFS or overall survival (OS) we assayed fresh and stored sera from 44 MM(25 F19 M, median age 55 years, 26 IgG, 13 IgA, 1 IgD, 1 IgE, 2 Bence Jones, 1 nonsecretory); patients who underwent autologous stem cell transplantation (ASCT). Patients were followed since ASCT to date of this report. Serum protein electrophoresis (SPE) and IFEwere performed using Hydrasys 2 apparatus (SEBIA) and antisera from the same company. Serum FLC and HLC (IgGκ/IgGλ, IgAκ/IgAλ) measurements were made on a Siemens BN^{TM} II nephelometer using polyclonal antisera assays (Freelite^{TM}, and Hevylite^{TM}, Binding Site,Birmingham,UK). Of the 44 patients in 26 (59%) after ASCT normalization of serum FLC ratio occurred. In all these 26 patients with normal FLC ratio SPE did not reveal monoclonal component and in 22 (84. 6%) of them also normalization of HLC ratio was noted but in 5 (19%) patientsIFE was still positive. Concordance of three tests (IFE negative, HLC normal, FLC normal) was found in 19 (73%) patients. Among 22 patients with normalization of HLC ratio in 3 (13%)IFE-was positive. In 21 of 44 (47%) patients after ASCT normalization of FLC ratio documented sCR. In this group 5 (28%) patients relapsed. Median PFS was 24 months (range 7-35). In the group of patients with CR, VGPR and PR (n=20) 12 (60%) patients progressed. Median PFS was 12 months (range 13-74). Three patients did not respond. Median follow-up period after ASCT of patients with sCR is 22 months (7-64), of patients with CR, VGPR and PR 25 months (5-108) and of non responders 9 months (3-25). So far, one patient died. One our patient with IgAλ MM, sCR after ASCT and FLCλ clonal escape at relapse and at that time with negative IgAλ IFE and normal IgA HLC ratio illustrates the importance of serum FLC elevation for early detection of clinical relapse, in the absence of any other clinical and laboratory finding. **Conclusions.** IFEis more sensitive that HLC and FLC assays in detecting residual disease. CR with normalization of the HLC and FLC ratios have impact on PFS but not OS.

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MULTIPLE MYELOMA RELAPSE TREATED BY BORTEZOMIB WITH CYCLOPHOSPHAMIDE AND PREDNISOLONE

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Background. Bortezomib monotherapy is effective in 27% of patients with relapsed and refractory multiple myeloma (MM), 43% of patients had relapses after 1-3 lines of chemotherapy. Adding dexamethasone to bortezomib therapy improves outcomes by 12-18%. Alkylating agents (melphalan, cyclophosphamide) significantly increase the effectiveness of bortezomib treatment.
Aims. Our aim was to assess the efficacy and the toxicity of the program BCP (bortezomib, cyclophosphamide, prednisone) in patients with relapsed MM.
Methods. From 05. 2007 till 02. 2012 43 patients at the age of 22 to 83 years (median 60 years), with relapsed MM were treated with BCP: 18 women (42%), 25 men (68%). All patients had previously received treatment, from 1 to 8 lines of chemotherapy (median 3), 8 patients had previously received bortezomib (19%), 10 patients (23%) of high-dose chemotherapy with autologous hematopoietic stem cells. BCP program included 8 cycles of induction: bortezomib 1. 3 mg/m^2 intravenously on days 1,4,8,11, cyclophosphamide 200 mg intramuscularly in days 1,4,8,11,14, prednisolone 1 mg/kg per os with 1 to 8 days of the course with a gradual reduction in dose and the treatment stop on 13 day. The cycle was renewed for 22 days from the beginning of the previous one. Then 3 cycles of maintenance: bortezomib 1. 3 mg/m^2 intravenously on days 1,8,15 and 22, cyclophosphamide 200 mg intramuscularly every 4 days, prednisolone 1 mg/kg per os 1 to 8 days of the cycle with a gradual reduction the dose and the complete withdrawal on day 13 of treatment. The cycle was renewed for 36 days from the beginning of the previous one. Patients received 1 to 14 cycles (median 7). Efficacy of the treatment was assessed in 42 patients according to the criteria of the International Myeloma Working Group (IMWG, 2006). Toxicity assessed according to the criteria of Common Terminology Criteria for Adverse Events (version 3. 0). **Results.** Overall efficacy was 67%, complete, very good partial, partial response were obtained in 12%, 17% and 38% of patients, respectively. The duration of observation was from 2 to 48 months (median 21), median overall survival was 30 months, median time to progression - 14 months. Adverse events included grade 3-4 thrombocytopenia (9%), neutropenia (9%), infections (19%), weakness (15%). In 12% of the cases developed herpes zoster. Peripheral neuropathy grade 2 was observed in 46% of patients, grade 3 - 7%. **Conclusions.** BCP is highly effective treatment for patients with relapsed MM and has moderate toxicity. It is recommended to use prophylactic antiviral drugs and dosage adjustment of bortezomib in patients with manifestations of polyneuropathy during therapy.

1527

INCREASED SERUM TRANSFORMING GROWTH FACTOR-BETA (TGF-BETA) IS RELATED TO LONGER TIME TO FIRST TREATMENT (TFT) AND OVERALL SURVIVAL (OS) IN WALDENSTRÖM'S MACROGLOBULINEMIA (WM)

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Background. TGF-beta has a tumor suppressor role by down-regulating cell proliferation. However, resistance to its effects was shown in malignant cells of B-lineage such as chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) cells while, in the same time, suppression of normal B-lymphocytes and plasma cells was observed, as expressed by decreased polyclonal immunoglobulin (Ig) production. There are no reports, to our knowledge, on serum TGF-beta levels in WM. **Aims.** To study any possible relationship between serum TGF-beta and parameters of disease activity and survival in a series of WM patients at diagnosis. **Patients and Methods.** 34 patients were studied (25 males, 9 females); median age was 64 years. Lymph nodes were palpable in 21% and spleen in 6%. Hb <11g/L was observed in 56%, PLT <140x10^9/L in 9%, serum albumin <3. 5 g/dL in 10%, beta2-microglobulin ≥ 5. 5 mg/L in 45%, bone marrow (BM) lymphoplasmacytic infiltration was ≥50% in 57% of patients. Median IgM level was 1900 mg/dL. 12% of patients were asymptomatic at diagnosis while 88% were symptomatic and required treatment. Median follow-up was 101 months. Median TFT was 5 months. Sera, drawn at diagnosis, were frozen and then retrospectively analyzed; sera from 24 healthy individuals (HI) were tested as controls. Serum TGF-beta measurements were done by ELISA (R&D, quantiquine) according to the manufacturer's instructions. Statistical analysis was performed by IPSS software, version 15v. **Results.** Median serum TGF-beta levels were 42000 pg/ml (range 6300-615000) in patients and 57500pg/ml in HI (range 30000-110000). No correlations were found between clinical and laboratory variables of disease and TGF-beta levels. Importantly, however, patients with TGF-beta levels above median presented a longer TFT than the others (p=0. 02) as well as a longer OS (p=0. 01). **Conclusions.** Our results of longer TFT and OS in WM patients with increased serum TGF-beta levels, suggest that WM lymphoplasmacytes remain sensitive to the suppressive effect of TGF-beta. Further studies are needed to confirm these results.

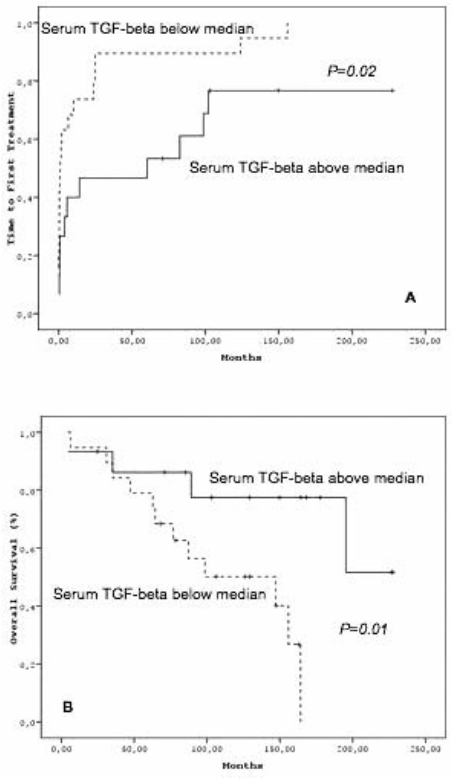

Figure 1.

1528

WITHDRAWN

1529

STUDY OF DOSE INTENSITY (DI) OF LENALIDOMIDA AS SALVAGE THERAPY IN PATIENTS WITH RELAPSE/REFRACTORY MULTIPLE MYELOMA. DIFERENCES OF RESPONSE AND OUTCOME ACCORDING TO DI

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Background. Lenalidomide is an inmunomodulatory drug used in the treatment of multiple myeloma (MM). Nowadays, treatment is maintained as long as the patient tolerates the drug and MM maintains response. Nevertheless, up to 40% of patients treated with lenalidomide suffer from grade 3-4 hematological toxicity being necessary dose reductions. Inmunomodulatory effect of lenalidomide is considered not to be related to dose. Until now there are no studies of the influence of dose intensity (DI) in quality and length of response. **Aims.** To compare the influence of DI of lenalidomide in response, progression free survival (PFS) and overall survival (OS) in patients with relapse MM treated with lenalidomide. **Materials and Methods.** DI of lenalidomide was calculated retrospectively in patients treated with lenalidomide for relapse or refractory MM. DI was calculated as median mg received per month at two points (from start lenalidomide to best response [1º DI] and from best response to end of treatment due to progression or death [2º DI]. In order to better comprehension, dose of lenalidomide is expressed as mg/day of 21-day/month cycle. 1ºDI was calculated in patients with a minimum of 2 cycles, and at least 4 weeks of treatment from best response was required for calculate 2ºDI. Patients with renal failure and primary refractory MM were excluded. **Results.** From January 2007 to December 2011, 71 patients were included in the study. Median age at lenalidomide treatment was 69 years old (40-85), median time from diagnosis to treatment with lenalidomide was 41 months (3-249), and half of the patients received at least 2 lines of treatments (1-6). Overall response rate (ORR)(≥PR) was 71,8% (18,3% CR, and 15,5% VGPR), 18 patients (28,2%) remain with stable disease. Median PFS and OS were 13 (3-50) and 16 months (5-60), respectively. PFS and OS since best response (PFSbr and OSbr) were also measured. Median PFSbs and OSbr were 6 and 12 months, respectively. Considering DI, median dose for 1ºDI was 18 mg/day/cycle (1,74-25). 47% of patients with 25 mg/day/cycle achieved CR vs. 32% for patients receiving lower doses (p:0,187), with shorter mean time to reach best response(18 vs. 33 weeks, p: 0,005). When we focused on 2ºDI, median dose was 11,93 mg/day/cycle (11,93-24,5). Interestingly, among patients receiving <15 mg/day/cycle vs. >15 mg/day/cycle a dramatic increase in PFSbr was observed (30 vs 6 months, respectively; p:0,025) and this was translated to an increased in OSbr (32 vs 14 months, respectively; p: 0,015) (Figure 1).

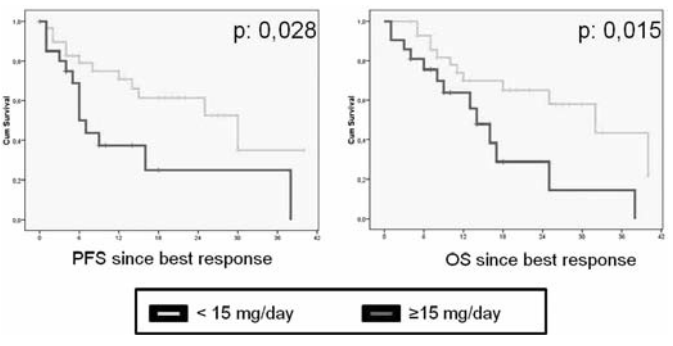

Figure 1. PFS and OS since best response.

Conclusions. For the best of our knowledge, this is the first study that correlates de dose intensity of lenalidomide with ORR and outcome in MM. We observed a trend toward better ORR was observed for patients receiving higher doses of lenalidomide, with shorter time to response. Nevertheless, once achieved best response, patients receiving lower doses of lenalidomide show higher PFS and this is translated into an increased in OS. The result of present study suggests a considerable inmunomodulatory role of lenalidomide in maintaining response. This surprisingly results should be prospectively confirmed.

1530

ANALYSIS OF THE NUMBER OF COLLECTED CD34+ CELLS AFTER DIFFERENT INDUCTION REGIMENS IN PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) remains the treatment of choice for patients with multiple myeloma (MM) eligible for this procedure. Until recently, the most commonly used induction therapy was the combination of vincristine, doxorubicin, and dexamethasone (VAD). Factors such as age, disease status, prior radiotherapy, treatment protocols, the mobilization regimen used and type of agent used for induction, especially alkylating agents, can influence the number of collected cells. The VAD regimen rarely results in failure to collect stem cells. Currently, several combinations of new drugs such as thalidomide, bortezomib and lenalidomide have been used for induction pre ASCT, with clearly superior results, as compared to VAD. However, there are few studies describing the impact of these new agents on the number of collected CD34+ cells, specifically for the CTD combination, which is widely utilized in our country. **Aims.** To analyze the number of CD34+ collected cells after three different induction regimens: VAD vs Cyclophosphamide + Thalidomide + Dexamethasone (CTD) vs Thalidomide + Dexamethasone (TD). **Patients and Methods.** A retrospective cohort of MM patients eligible for ASCT who underwent different induction regimens (VAD, TD and CTD) was studied. Data were collected from patients treated from January 2007 to July 2011. The appropriate cell number collection was greater than or equal to 2 x10^6/Kg. Patients with MM who underwent induction with VAD, TD or CTD were included. Mobilization was performed with the GCSF alone or GCSF+cyclophosphamide. We excluded patients who had received prior to ASTC more than six cycles or regimens other than those above. Statistical analysis was performed with SPSS 15. 0, through comparative analysis with ANOVA, Student's t test, Welch test and Tukey Post Hoc test. **Results.** A total of 80 MM patients submitted to ASCT was elected. Seven patients were excluded, with 73 analyzable patients remaining. The patients were 56% male. The median age at diagnosis was 55 years old (28-68). The immunoglobulin subtypes at diagnosis were: IgG = 50%, IgA = 23%, 19% = light chain, IgM = 1. 4% and non-secreting = 3%. The Durie-Salmon staging was: IIIA+B=88% and IIA+B=12%. The numbers by the induction + mobilization scheme were: VAD+(Cy+GCSF)=17 cases, VAD+GCSF=10, CTD+GCSF=14 and TD+GCSF= 31. The median number of induction cycles was three for VAD and four for TD and CTD. The median of collected CD34+ cells was: VAD + (Cy + GCSF)= 5. 9x10^6/Kg; VAD+GCSF=5. 28 x10^6/Kg, CTD+GCSF=2. 75x10^6/Kg and TD+GCSF=2. 85x10^6/Kg. The median number of collection apheresis for VAD+(Cy + GCSF), CTD and TD was once. The group that received VAD+GCSF required a median of 1. 5 times. The general analysis of the number of collected CD34+ cells showed differences among the groups, the VAD group having the advantage of a much larger number of collected cells (p <0. 0001). However, when analyzing the collection of CD34+ cells among the different groups that used only GCSF as mobilization, there were no differences (p=0,146). **Conclusions.** The CTD combination as induction followed by GCSF mobilization alone allowed for the collection of CD34+ cells in sufficient quantities to perform one ASCT, which is the most readily accessible treatment in our country.

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EXTRAMEDULLARY RELAPSE OF MULTIPLE MYELOMA: DISCORDANT RESPONSE TO TREATMENT OR PROGRESSION IN PATIENTS WITH MULTIFOCAL LESIONS

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Background. Extramedullary disease (EMD) is an uncommon manifestation of Multiple Myeloma (MM), characterized by high-grade histology, aggressive biological behaviour and very short survival. EMD may occur in MM patients with relapsed disease more often than in patients at diagnosis. Its incidence has increased during the last years, probably due to the prolongation of patients' survival and to the availability of more sensitive imaging techniques. Data focusing on the response to treatment and the outcome of EM MM according to the involved body sites are limited and controversial. **Aims.** The purpose of this study was to assess the different pattern of response to treatment between the involved sites. We report hereby a series of 15 MM relapsed patients with more than one EM lesions and that showed discordant response to treatment or progression from site to site. **Methods.** We retrospectively reviewed all relapsed MM patients observed at our Institution, who received at relapse Bortezomib based regimens (BBRs) and had a follow-up time of at least 6 months. In 15 cases (5. 8% of the 256 MM patients observed at our institution over a 7-year period) the relapse presented with multifocal lesions with involvement of soft tissues surrounding the skeleton in 65% of cases (paravertebral soft tissues, soft tissue arising from the skull or from the chest wall) and involvement of other sites (airways, skin, subcutaneous tissues, lymph nodes, pericardium, pleura, gastrointestinal tract, liver, kidney, abdominal pelvic masses, muscles) in the remaining 35%. The median number of EM sites per patient was 3 (range 2-5 sites per patient). Diagnoses were made from biopsy and/or clinical follow-up findings. **Results.** After 6 cycles of treatment, the EM masses showed discordant response to treatment or progression from site to site whereas the monoclonal protein (M protein) decreased in all cases. Masses arising from the bone with a soft tissue component showed a response to treatment (until complete disappearance in some patients) in 70% of cases; masses involving other sites showed a change in the size in 45% of cases. Cutaneous localizations had only a transient response followed by a rapid progression and the onset of several subcutaneous nodules. **Conclusions.** According to definition of EMMM, EM masses must not have risen from any bone, whereas masses arising from the bone with a soft tissue component should be defined extraosseous. Our case series indicates that, after uniform treatment (BBRs), EMMM shows discordant response to treatment or progression from site to site. Moreover, the response to treatment may differ between various involved sites in each patient with multifocal EM lesions. In our case series, when EMMM involves soft tissues surrounding the axial skeleton, the effect of the treatment is mainly better than in other cases. Finally, the response and the outcome are very poor when EMD is represented by skin involvement. Our data suggest that the progression of myeloma cell homing in different tissues may affect the response to therapy and the EM myeloma cells may become more tolerant to bortezomib in some sites rather than in others.

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EFFICACY OF THE COMBINATION THERAPY OF LIPOSOMAL ADRYAMICIN, BORTEZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE (ABCD) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background. Bortezomib has shown significant activity in myeloma. In this multicenter trial, we assessed for the first time the combination of bortezomib, doxorubicin cyclophosphamide, and dexamethasone (ABCD) in the treatment of relapsed/refractory myeloma. **Patients and Methods.** Twenty-four patients were treated for a median of four 28-day cycles (1-6). Median age was 68 years (range, 51-78 years). Three had extra-medullar disease. All patients had been already treated with a median of 3 previous lines of treatment (range, 2-6): 38% were resistant to previous therapies and 62% were relapsed. Twenty-five percent of patients had undergone prior autologous transplantation, 25% prior anthracycline and 42% prior bortezomib-based regimens. Bortezomib was given at dosage 1. 3 mg/m2, and dexamethasone 40 mg I. V. on days 1, 4, 8, 15 plus liposomal doxorubicin 20 mg I. V. on days 1 and 15, and cyclophosphamide 100 mg per os for 15 days. **Results.** At least a partial remission (reduction M-protein > 50%, according to International Myeloma Working Group Criteria) was achieved in 50% of patients, including 8% of complete remissions and 21% of very good partial remissions (reduction M-protein > 90%, according to International Myeloma Working Group Criteria). Side effects were predictable and manageable, with prominent hematological (31% any grade), including grade 3-4 thrombocytopenia (9%), grade 3-4 anemia (17%). Non hematological toxicity affected 32% of administered cycles and included gastrointestinal disturbances (54%), peripheral neuropathy (8%) and infections (8%). After a median follow up of 22. 6 months, one-year progression-free survival was 27% and overall survival from the start of ABCD was 33%. Median time to progression was 8. 4 months. **Conclusions.** ABCD is an active salvage therapy with manageable toxicity in patients with relapsed/refractory myeloma.

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DESCRIPTION OF A RARE AND NOVEL DISEASE: TEMPI SYNDROME

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We have previously described the TEMPI syndrome, a multisystem disease characterized by the pentad of Telangiectasias, elevated Erythropoietin and erythrocytosis, Monoclonal gammopathy, Perinephric fluid collections, and Intrapulmonary shunting. Here, we expand on the original description of the TEMPI syndrome and provide new data towards its successful treatment. Together, we have identified and are following four patients that demonstrate the five characteristic features. These patients developed their first symptoms of erythrocytosis in their thirties and forties and these symptoms have been slowly progressive. Telangiectasias are most prominent on the trunk, arms, hands, face and oropharynx. Tests for liver dysfunction and genetic screening for hereditary telangiectasia were negative, the VEGF level was normal. All patients had erythrocytoisis at the initial presentation with hematocrit values up to 64% and they were treated with phlebotomies. The serum Erythropoietin levels were elevated at diagnosis and the values rose to levels above 5000 mU/ml. A cause could not be determined. Imaging, hemoglobin oxygen affinity tests, hemoglobin electrophoresis, JAK2 and HIF-2alfa exon 12 gene sequencing did not elucidate the pathogenesis. An IgG Monoclonal gammopathy was identified: three patients had an associated kappa light chain and one a lambda light chain. The bone marrow biopsies revealed fewer than 10% plasma cells in three patients, consistent with a diagnosis of monoclonal gammopathy of undetermined significance and there were 30% plasma cells in the bone marrow of the last patient who had been followed fourteen years for erythrocytosis. There was no end-organ involvement. The progressive accumulation of bilateral Perinephric fluid collections is a striking and unique feature of the syndrome. The serous fluid was clear, low in protein, with few leucocytes and without cholesterol or triglycerides. Microscopic Intrapulmonary shunting with hypoxia was present in three patients and slowly progressed. Pulmonary hypertension was not detected with right heart catheterization. We speculate that the TEMPI syndrome is the end stage of a spectrum of associations that are reported in the literature: erythrocytosis and monoclonal gammopathy and monoclonal gammopathy and perinephric fluid collections. We hypothesize that the paraprotein is involved in the pathophysiology of the TEMPI syndrome. We cloned the heavy and light chain variable regions corresponding to the monoclonal protein of one of the patients, introduced them into an expression construct and have generated a monoclonal antibody that we are currently testing for its specificity. Treatment with the proteasome inhibitor bortezomib is ongoing in all patients after the dramatic resolution of all of the TEMPI syndrome characteristics was observed in one of them. We bring this novel syndrome to the attention of the hematology community hoping to identify more cases.

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INCREASED BONE MARROW TOTAL VASCULAR AREA (TVA) CORRELATES WITH A MORE AGGRESSIVE DISEASE IN WALDENSTRÖM'S MACROGLOBULINEMIA (WM) PATIENTS

?? Tzenou[1], G Levidou[2], N Kavantzas[2], K Xirokosta[1], D Maltezas[1], P Korkolopoulou[2], E Koulieris[1], K Dimou[1], C Kalpadakis[3], T Vassilakopoulos[3], M Angelopoulou[3], P Panayiotidis[1], G Pangalis[3], E Patsouris[2], MC Kyrtsonis[1]
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Background. Angiogenesis plays an important role in tumor progression by numerous mechanisms among which is the physical expansion of endothelial surface that gives tumor cells more opportunities to enter the circulation and spread. Very few studies have explored this issue in WM. **Aims.** To study the possible role of increased bone marrow (BM) neoangiogenesis in WM by evaluating microvessels in immunostained sections by computer image analysis. **Methods.** Angiogenesis was evaluated by CD34 immunostaining in BM trephine biopsy specimens from 36 WM patients at diagnosis. A digital camera connected to a bright field microscope was used. Pictures were examined with a standardized program to quantify the proportion between stained and total areas and to assess the number of microvessels (image analysis software). Two main parameters were evaluated, microvessel density (MVD) and total vascular area (TVA). Symptomatic patients (84%) received treatment while the others (16%) were regularly followed. Median time to first treatment (TFT) was 2 months while median overall survival was 89 months. Results of microvessels immunostaining were compared to patients and disease variables such as patients' age, sex, presence of lymphadenopathy or organomegaly, anemia, thrombocytopenia, increased LDH, beta-2 microglobulin, IgM levels and percentage of BM infiltration. Statistical analysis was performed by IPSS software, version 15v. **Results.** MVD ranged from 7 to 393 (median 32); TVA ranged from 2681 to 69160 μm^2 (median 27762). Patients with TVA above median had a statistically significant shorter TFT and OS (p=0. 03 for both) than the others. MVD failed to show correlation with TFT and OS. No correlation was found between TVA, as well as MVD, and other clinical and laboratory disease parameters. **Conclusions.** Increased TVA may prove a powerful predictor of TFT and OS in WM. Results need to be confirmed in larger studies.

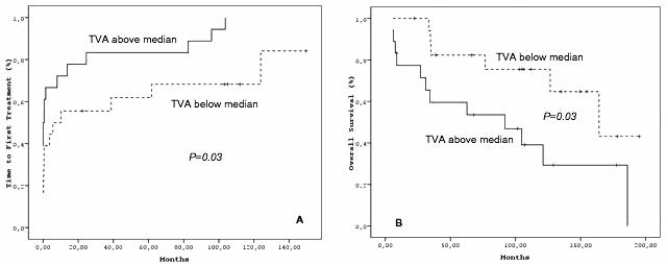

Figure 1.

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GENETIC POLYMORPHISMS FOR SELECTED CYTOKINES AND TOLL-LIKE RECEPTORS IN MULTIPLE MYELOMA

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Backround. Myeloma multiple (MM) is a clonal neoplastic disease characterized by proliferation of malignant plasma cells in bone marrow which secrete monoclonal immunoglobulines. Stromal cells in bone marrow, which secrete cytokins such as interleukin 6, interleukin 10, tumor necrosis factor α and lymphotoxin α, play important role in pathogenesis of MM. The role of Toll-like receptors in pathogenesis of MM has been recently studied. Numerous studies signalize the association between single nucleotide polimorphysm (SNP) and risk for developing MM, prognosis and therapy response. **Aims** The goal of this study is to determine if there is association between different polymorphisms of genes for cytokines and toll-like receptors and clinical-laboratory characteristics of disease, and to acess whether any of genotypes was associated with increased risk for development MM or affect survival of patients. **Methods.** 45 patients and 115 healthy individuals were inrolled in this study. SNP for studied cytokins were identified with polymerase chain reaction followed by restriction fragment length polymorphism. **Results.** There was no significant statistical difference regarding distribution of genotypes between both groups except for GC genotype of IL6G(174)C polymorphisms, which was significantly more presented in group of patients. Logistic regresion method showed that the same genotype was independent risk factor for developing MM (p<0. 05, OR 2. 465, 95% CI 1. 105-5. 497). No correlation was found between other genotypes and susceptibility to MM. Tested polymorphisms didn't affect survival. The correlation between certain genotypes and clinical-laboratory characteristics of MM was found. **Conclusions.** Genetic factors may impact cell functioning, MM characteristics and therapy responce and represent a risk factor for MM developement

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F-FDG PET/CT FOR DETECTION AND LOCALIZATION OF SOLID NEOPLASMS IN PATIENTS WHIT MONOCLONAL GAMMOPATHY UNDETERMINED SIGNIFICANCE (MGUS

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Background. A review of the literature shows discordant data on the association between monoclonal components and solid neoplasms. **Aims.** we want demonstrate possible usefulness of F-FDG PET/CT for detection of solid neoplasms in MGUS. **Methods.** 60 patients (M: 34, F: 26, age range: 65-78yrs) with MGUS diagnosed by standard criteria have been included in this perspective study. 30 patients showed a serum IgG (16 κ; 14 λ;), 6 an Ig M (4 κ; 2 λ), and 24 an IgA (10 κ; 14 λ;) monoclonal component. Total body PET-CT was performed in all patients, in addition to a standard radiographic scan of all bones. **Results.** An associated solid tumour was found in 16/60 cases (26,6%; 10M, 6F). An IgA monoclonal component has been detected in 10/16 (62,5%; 6M, 4F) patients. In this subgroup the associated neoplasms were: colorectal adenocarcinoma (6), non-small cell lung cancer (2), and clear cell renal carcinoma (2). Colorectal adenocarcinoma and infiltrating ductal breast carcinoma were respectively diagnosed in five patients presenting IgG monoclonal component. At last an IgM paraprotein was detected in a colorectal adenocarcinoma patient. **Conclusions.** Total body PET-CT allowed us to diagnose different neoplasms, in early stage, in patients with MGUS. We showed how, in our limited population, PET-CT is comparable in sensibility and specificity to complete bone standard radiography for the study of bone disease. Moreover, this diagnostic technique allowed the early detection of solid tumors. Interestingly, in 10/16 cases (62,5%) the associated neoplasia was localized at the bowel, and in this subgroup the monoclonal component was Ig A in 6/10 cases. This study, even if conducted on a limited population, suggests that PET-CT could be usefully considered in the diagnostic algorithm of MGUS in order to discover eventually associated solid neoplasms.

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PERIPHERAL BLOOD STEM CELL MOBILIZATION USING G-CSF ALONE FOR MM WITH THE MINIMAL RESIDUAL DISEASE COMPARED TO CYCLOPHOSPHAMIDE PLUS G-CSF

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Background. Autologous stem cell transplantation (ASCT) for multiple myeloma (MM) offers higher response rates and improved survival compared with conventional chemotherapy. However, the best method for peripheral blood progenitor cell (PBPC) mobilization in patients with MM remains controversial. **Aims.** We analyzed the efficacy of PBPC collection, complication, and the transplant outcome of G-CSF alone, compared to high-dose cyclophosphamide (HD-CY) + G-CSF as the regimen of PBPC collection for autologous transplantation in 54 patients with MM. **Methods.** In group I (n = 37), G-CSF, 10 μg/kg/day s. c., was used alone until the last day of collection, starting consecutive aphereses on the 5th day. In group II (n = 17), HD-CY, 4 g/m^2 i. v., was administered followed by G-CSF, 10 μg/kg/day s. c., until the end of collection, starting the leukaphereses after hematological recovery (WBC >5,000 x 10^6/L). **Results.** Both groups were comparable for age, sex and clinical prognostic features as well as previous therapies. However, in patients mobilized by HD-CY + G-CSF, the percentage of plasma cell in bone marrow prior to mobilization was significantly higher. (7. 15% vs. 1. 0%, p=0. 004). Median total yields of CD34+ cells had not significant differences between two groups (7. 86 x 10^6 cell/kg vs. 9. 0 x 10^6 cell/kg, P=0. 35). Successful collection more than 5. 0 x 10^6 CD34+ cells/kg and not failed collection of at least 2. 0 x 10^6 CD34+ cells/kg were achieved in similar proportions in the two groups(64. 9% vs. 64. 7%, P=0. 991 and 91. 9% vs. 76. 5%, P=0. 189). The significant differences of yields on day 1 ≥ 2. 0 x 10^6 CD34+ cells/kg were not observed either (56. 8% vs. 64. 7%, P=0. 581). Hospitalization for peripheral blood stem cell mobilization was longer in group II (9days vs. 18days, p<0. 001) and the treatment-related toxicity was greater in this group: 8 patients (47%) developed fever requiring antibiotics during the neutropenic period after HD-CY and seven (41%) patients required transfusion support. Among 54 patients, 44 underwent ASCT (14 and 30 belonged to the group I and II, respectively). After stem cell infusion, no significant differences of the median time to neutrophil engraftment (>0. 5 x 10^9/l) and platelet engraftment (>50 x 10^9/l) were observed between both groups (10days vs. 10days and 15days vs. 14days, respectively). Post transplant responses were not different in the two groups, with 70. 0% of patients improved or retained CR status after ASCT in the group I and 78. 6% in the group II (p=0. 722). Overall and progression-free survivals were similar in both groups. The overall survival were 80% and 71% (95% CI, ± 11% and ± 14%, p=0. 282) at 2 years. The progression-free survivals were 50% and 46% (95% CI, ± 16% and ± 11%, p=0. 745) at 2 years. **Conclusions.** These data suggest that adequate CD34+ cell collections can be achieved with G-CSF alone and G-CSF alone is cost-effective with less toxicity and with simplification of the procedure. G-CSF alone as mobilization regimen is reasonable at least in patients who were confirmed lower residual disease with bone marrow study prior to mobilization.

1538

EFFICACY AND SAFETY OF BORTEZOMIB-BASED RETREATMENT IN MULTIPLE MYELOMA (MM) PATIENTS RELAPSING AFTER BORTEZOMIB-CONTAINING FIRST-LINE THERAPY: A RETROSPECTIVE STUDY

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Background. Multiple myeloma (MM) follows a relapsing course; hence, there is a need for multiple active therapeutic options after first-line treatment, including the possible re-use of front-line agents. Bortezomib-based combination therapies are increasingly used as first-line MM therapy, so there is a need for an assessment of the efficacy and safety of bortezomib retreatment in MM patients. **Aims.** This international, multicentre, non-interventional retrospective study aimed to assess the efficacy and safety of bortezomib-based retreatment in MM patients relapsing after bortezomib-containing first-line therapy. **Methods.** Eligible patients were aged ≥18 years with a positive diagnosis of MM; patients had previously responded (at least partial response [PR]) to bortezomib-containing first-line therapy (in the transplant or non-transplant settings), relapsed following a treatment-free interval (TFI) of at least 6 months; and received at least 3 cycles of bortezomib retreatment as second-line therapy, which had been completed at least 2 months prior to study start. Bortezomib retreatment dose and schedule were selected by the treating physician. Response was assessed by EBMT or IMWG criteria. **Results.** A total of 35 patients across 15 centres were identified; median age at time of diagnosis was 62. 7 years (range 34. 2-84. 0). Most patients received bortezomib in combination with dexamethasone (20 and 18 for first-line and retreatment, respectively). Median follow-up from bortezomib retreatment was 1. 2 years (range 0. 2-3. 2). Patients received a median of 4 cycles (range 1-9) as first-line treatment and 4 cycles (range 3-13) as retreatment (Table 1).

Table 1.

	Bortezomib first-line treatment	Bortezomib retreatment
Median treatment duration, cycles (range)	4 cycles (1–9)	4 cycles (3–13)
Best response		
CR	12	5
VGPR	8	8
PR	15	15
SD	–	5
PD	–	1
Unknown	–	1
Median time to best response, months (range)	2.1 (0.4–24.0)	2.3 (0.7–18.6)
Median duration of response, months (range)	30 (9–59)	15 (1–54)
Median PFS, months (range)	31.3 (11–60)	14.5 (1–56)

Best response to bortezomib as first-line therapy included 12 complete response (CR), 8 very good partial response (VGPR), and 15 PR; median time to best response was 2. 1 months (range 0. 4-24. 0), median duration of response was 30 months (range 9-59), and median progression-free survival (PFS) was 31. 3 months (range 11-60). Best response to bortezomib retreatment included 5 CR, 8 VGPR, 15 PR, 5 stable disease (SD), 1 progressive disease (PD), and 1 unknown, to give an overall response rate of 80%, including

a ≥VGPR rate of 37%; median time to response (n=28) was 2. 3 months (range 0. 7-18. 6), median duration of response was 15 months (range 1-54), and median PFS was 14. 5 months (range 1-56) (Table). Eleven of the 29 patients alive at study end (database lock: Dec 21, 2011) have not experienced disease progression, and 20 have not received subsequent therapy. Sixteen patients (46%) experienced at least one adverse drug reaction (ADR), including 10 patients during first-line treatment and 10 during retreatment; most ADRs were mild or moderate (48% and 33%, respectively). The most common ADRs were anaemia (n=4), peripheral sensory neuropathy (n=4; 3 during first-line treatment and 1 during retreatment), diarrhea, neutropenia, and thrombocytopenia (each n=3). **Conclusions.** Bortezomib retreatment, following relapse after bortezomib-containing first-line treatment, is associated with high-quality responses and prolonged PFS in MM patients with adequate first-line responses.

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BORTEZOMIB, RESPONSE AND RETREATMENT IN 1ST, 2ND, 3RD AND 4TH LINE OF TREATMENT IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Bortezomib as single drug or in combinations is today an established therapy of multiple myeloma (MM) both in 1st line and as relapse medication. **Aims.** To assess the efficacy of Bortezomib in first and later lines in treatment hierarchy of MM and to assess the responses in re-treatment situations. **Methods.** Patients with symptomatic MM were selected from our institute between January 2000, and December 2010. The study included 243 consecutive patients who underwent treatment with Bortezomib in any line and out of those 119 (49%) were treated with an autologous stem cell transplant. As a number of patients were treated with Bortezomib at more than one treatment line, the total amount of Bortezomib treatment lines were 277; 77 (28%) in 1st line, 118 (43%), 50 (18%) and 32 (12%) in 2nd, 3rd and 4th treatment line, respectively. **Results.** In 1st line treatment 48% of the patients achieved nCR, 17% VGPR, 22% PR and 13% NR with an ORR of 87%, the median time to progression (TTP) was 18 months and median overall survival time (OS) was 51. 7 months. In 2nd, 3rd and 4th treatment line, the ORR was 77%, 68% and 57%, TTP was 9, 6 and 6 months respectively and OS was 39. 2, 24. 3 and 16. 8 months respectively. 8 patients underwent treatment with Bortezomib in 1st and 2nd line. The overall response rate (ORR) was 75% in 1st line and 62% in 2nd line. 13 patients received Bortezomib in 2nd and 3rd line. The ORR was 92% in 2nd line and 85% in 3rd line. 9 patients received Bortezomib in 3rd and 4th line. The ORR was 100% in 3rd line and 89% in 4th line. **Conclusions.** Bortezomib is known to be a relatively safe drug with good responses when used in 1st and 2nd line treatment of MM. We have shown that it is possible to get good responses even in a heavily pre-treated population. We have also shown that patients may get a good response with Bortezomib even if they have been treated with Bortezomib in a previous treatment line. Re-treatment is otherwise thought to be hindered by drug-resistance, but our results indicate that it may not be a major issue with Bortezomib.

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TEACHING OLD DRUGS NEW TRICKS: RATIONALE FOR THE REDEPLOYMENT OF VALPROATE, AN ANTI-CONVULSANT, AND NICLOSAMIDE, AN ANTI-HELMINTHIC AGENT, AS A COMBINATION THERAPY AGAINST MULTIPLE MYELOMA

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Background. Multiple Myeloma is a plasma B cell neoplasm which, despite recent advances in treatment, remains incurable. If untreated, most patients die from infection, renal impairment or anaemia within months of diagnosis. Current best treatments offer an overall 5-year survival of only 36% and relapse with resistant disease is a major cause of death. However, current treatments are associated with significant comorbidites rendering the majority of older patients, >70% of MM cases, ineligible for intensive treatment regimens. **Aims.** The aim of our study was to screen a panel of 100 licensed, well-tolerated drugs from the BNF for anti-MM activity. We demonstrate here that valproate and niclosamide (VaN) combination therapy has potent selective anti-MM activity that is mediated, in large part, through targeting the mitochondria of the tumour cells. **Methods.** MM cell lines and primary MM bone marrow samples were treated in vitro with valproate and/or niclosamide. Cell viability was determined using annexin V/propidium iodide staining and CellTiter blue assay. FLC levels were measured by Luminex. Mitochondrial function and health and oxidative stress levels were assayed using a panel of flow cytometric and immunofluorescence assays. QRT-PCR, immunoprecipitations and immunoblotting were used to measure levels of key oxidative stress response genes. **Results.** Niclosamide demonstrated potent anti-MM activity against cell lines and primaries and also induced a significant decrease in FLC secretion. Valproate had very little anti-MM activity on its own, however significantly potentiated the actions of niclosamide in vitro against MM cell lines and primaries when used as a combination treatment (VaN). Importantly, VaN had little impact on CD34+ blasts in vitro. MM cell death with VaN was associated with markers of both apoptosis and autophagic cell death. Our data shows that in MM cells niclosamide uncoupled oxidative phosphorylation causing depolarisation of the mitochondrial membrane, production of mitochondrial superoxide and reduced ATP production. Production of mitochondrial superoxide was significantly enhanced by valproate indicating that valproate might be disabling the anti-oxidant response of the MM cells. Valproate functions as a histone deacetylase inhibitor (HDACI) but non-histone proteins have also been reported to be regulated by acetylation state. Indeed, the majority of enzymes involved in metabolism have been shown to be acetylated. We demonstrate here that valproate in combination with niclosamide, enhanced mitochondrial superoxide production by regulating mRNA, protein levels and acetylation status of key genes involved in the antioxidant response including SIRT3, a mitochondrial deacetylase, and manganese superoxide dismutase (SOD2) a key regulator of mitochondrial superoxide levels. Importantly, no induction of mitochondrial superoxide was observed in normal donor cells. **Summary and Conclusions.** Our study demonstrates that valproate and niclosamide (VaN), at clinically achievable concentrations, have potent anti-MM activity. We demonstrate that this activity is tumour selective and is mediated by the generation of oxidative stress levels that are lethal for the MM cells. These data taken in the context of the known safety profiles of valproate and niclosamide, provide rationale for their use as a combination therapy in the treatment of elderly and relapsed and/or refractory MM.

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BORTEZOMIB IN COMBINATION WITH MELPHALAN AND PREDNISONE (MPV) AS FIRST LINE THERAPY IN FRAIL ELDERLY PATIENTS AFFECTED BY MULTIPLE MYELOMA

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Background. Bortezomib has proven to be safe and effective in MM patients not only as monotherapy but also given in combination with cytotoxic agents. Bortezomib-based combination regimens have induced clinical benefits with manageable toxicities and may ultimately lead to improvement in the duration of response and survival of patients in the first-line setting. The use of bortezomib, in combination with melphalan and prednisone, has shown remarkable benefits, especially in elderly frail patients. **Aims.** We evaluated the efficiency and safety of bortezomib in combination with melphalan and prednisone (MPV) as a starting regimen for the treatment of elderly frail patients affected by MM. **Methods.** In our institution we are following 28 elderly patients with stage II/III MM (15 Fand13 M, median age: 75 years, r. 67-85 years). All patients were not eligible for aggressive treatment protocols because they had, at diagnosis, one or more comorbidities. As first-line treatment all patients received Melphalan and Prednisone plus Bortezomib chemotherapy (Melphalan 8 mg/sqm p. o. d. 1, 2, 3, 4; Prednisone 75mg p. o. d. 1, 2, 3, 4; Bortezomib 1,3 mg/sqm i. v. d. 1, 8, 15, 22 every 36 days). **Results.** At a clinical re-staging after four courses of therapy a reduction of M-component > 50-75% was recorded in 22 patients while the remaining patients was in steady disease (SD). Thereafter all patients received further four courses of therapy. At the end of treatment 6 out of 28 patients achieved CR and the remaining showed VGPR or PR. At the present, (month +18) only two patients show a progression disease, while six patients are in CR and the remaining in PR or VGPR. **Conclusions.** Our results suggest that the combination of melphalan-prednisone-bortezomib is effective and well tolerated in the treatment of MM in elderly “frail”patients. The once-weekly dosing regimen of bortezomib did not cause adverse effects, particularly the neurological. Although there are several published data on the activity of the therapy based on the combination melphalan-prednisone-bortezomib, little is still known about the improvement in the duration of response and survival of elderly patients in the first or second line therapies.

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COMBINATION THERAPY BASED ON BORTEZOMIB FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background. Novel drugs, such as bortezomib, have significantly improved the response rates in multiple myeloma (MM), but little has been reported on bortezomib-based therapies in Chinese patients. **Aims.** Here we report our results with combination therapy based on bortezomib in the Chinese population and investigat the efficacy and safety of Bortezomib-based therapies in previously untreated MM patients. **Methods.** Between 1st Feb. 2006 and 31st Dec. 2010, 102 consecutive newly-diagnosed patients with median age 59 years (range 31-86 years) with symptomatic MM were treated with combination therapies based on bortezomib including bortezomib plus dexamethasone (BD) and the triplet combinations of BD with adriamycin (BAD), cyclophosphamide (BCD), thalidomide (BDT). All patients received a median of three cycles of therapy (range 1-5). The IMWG criteria was used for response evaluation and toxicities were evluated according to the NCI Common Toxicity Criteria version 3. **Results.** The overall response rate (≥ partial response, PR) of all the patients was 85. 3% (including 27. 5% very good partial response (VGPR) and 23. 5% complete response/near complete response (CR/nCR). The median PFS for each arm was 12. 0 to 15. 0 months and without significant difference between the arms while the median OS for BDT arm was 35. 0 months, the median OS for other arms were not reached. The frequently observed toxicities were neutropenia, thrombocytopenia, fatigue, infection, herpes zoster, and peripheral neuropathy. Anti-viral agent acyclovir significantly reduced the incidence of herpes zoster (P =0. 004). A significant trend towards grade 2 or 3 peripheral neuropathy during BDT therapy was observed compared to the other groups (P =0. 028). **Conclusions.** Our experience indicated that combination chemotherapy of triplet combinations were superior to BD. Serious Adverse events were rare in the Chinese patients with MM who received bortezomi-based chemotherapy.

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CENTRAL SYSTEM NERVOUS MULTIPLE MYELOMA (CNS MM) RELAPSE/PROGRESSION FEATURES: A RETROSPECTIVE MULTI-CENTER STUDY

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Background. SNC MM relapse, defined as the detection of malignant plasma cells in the cerebrospinal fluid or in the intracranial sanctuary, is a rare event (1%) with a worse prognosis. To date the pathogenesis mechanisms remain unclear and the role of novel agents, intensive therapies, the prolonged overall survival and molecular basis are understood. **Aims.** In order to define the incidence, features and prognosis of CNS MM relapse this multi-center study was conducted. **Patients and Methods.** We revised 698 consecutive MM diagnoses admitted at 7 centers of GIMEMA MM Lazio from January 2006 to January 2011. **Results.** Among 6987 pts(1%),5 males, 2 females- median age 62y (49-67) had isolated CNS MM relapse. At MM diagnosis of these 3 were IgG k, 3 IgA (2 k, 1 λ), 1 IgD λ, 5/7 had D-S stage ≥II,2 ptsISS ≥3 and normal creatinine level in 5/7, BJ was positive in3 pts. BM involvement showed plasma cell < 10% in 2 cases. Baseline cytogenetics revealed high risk features in 2/2 pts studied. The CNS MM relapse sites included: brain in3 pts, spinal cord in3 ptsand leptomeningeal involvement in1 pt, respectively. In 3 cases early CNS relapsed occurred after first line therapy, in 2 after second and third-line therapy, respectively. At CNS MM occurrence relapse bone marrow infiltration was absent in 4/7 pts. Only in 2 cases B2Mg was >5, whereas elevated LDH levels has been detected in 4/7 pts. All the have been treated. 4 ptsreceived novel agents as bortezomib (2), lenalidomide (2), radiation therapy (3), conventional chemotherapy (1) without response in 6/7 cases. Median overall survival from MM diagnosis was 21 months (9-61) and from CNS MM 6 months (0,7-12) with only three patients being alive at 33, 37 and 60 months after MM. **Conclusions.** Novel agents have been proven to prolong OS and PFS in patients with MM. Nevertheless in the last years a prolonged follow up showed an increased atypical extramedullary relapses with a CNS involvement indicating that neoplastic plasma cells may display remarkable resistance to intensive conditioning regimens and exhibit immune escape mechanisms. Malignant and high proliferative plasma cell may migrate trough the hemato-encephalic barrier in the initial phases of disease and preserved from endovenous therapy may proliferate. Further elucidation of the factors predisposing patients with high-risk disease features to CNS MM involvement is needed.

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COMPLICATIONS LINKED TO TOTALLY IMPLANTABLE CENTRAL VENOUS DEVICES IN CHEMOTHERAPY OF MULTIPLE MYELOMA: A CASE SERIES OF 142 PATIENTS

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Background. There are few references to the complications associated with totally implanted devices for chemotherapy in patients with multiple myeloma(MM). The necessity to maintain prolonged treatment may involve more frequent complications. **Aims.** We aimed to evaluate the complications of totally implanted devices, Port-a-Cath (PAC), used for chemotherapy in MM patients. **Patients.** Prospective observational study of MM patients treated with chemotherapy through PAC in our department (1995-2007). Antibiotic prophylaxis with cefazoline was prescribed before PAC implantation. The device was maintained indefinitely during treatment, unless complications requiring its removal occurred (infection, thrombosis or dysfunction). Primary thromboprophylaxis was not routinely prescribed. The diagnosis of PAC infection was based on in differential growth blood cultures taken from PAC versus peripheral vein, when PAC culture was positive two or more hours earlier than the peripheral culture, or a positive culture of exudate from the pocket was obtained. Associated thrombosis was diagnosed by ultrasound or computerised tomography scan . Upon diagnosis of PAC infection, systemic antibiotherapy and antibiotic lock was prescribed and PAC maintained unless clinical deterioration of the patient or pocket infection occurred, making removal of the device necessary. Informed consent was obtained in every patient. Statistical **Methods.** Fisher exact test, Student t, log-rank test, Kaplan-Meier survival tables and Cox regression multivariate binary logistic model. **Results.** 155 implanted PAC in 142 patients (55% male and 45% female) were included for analysis. Median age was 67 years (27-84). Subtype of monoclonal immunoglobulin: G in 46. 5%, A in 27. 7%, D or light chain in 25. 8%. The median duration of each PAC was 20. 9 months (0. 5-108); the median duration of chemotherapy by PAC 9 months (0. 3- 42). Twenty nine infections of PAC (27 bacteremia and 2 pocket infections) were detected in 25 patients (0. 2 /1000 days). Coagulase negative Staphylococcus(CNS) (43. 7%) was the most frequently isolated microorganism, followed by Gram negative rods (25%) and other Gram positive cocci (14. 3 %). Antibiotic lock along with systemic antibiotics was prescribed in 18 infections (62%), achieving the maintenance of the device in 17 (94%). PAC associated thrombosis was detected in three patients (0. 02/1000 days). The device was removed in 31 patients (20%): in 13 patients (8. 3%) because of infection and in the remaining because of dysfunction or end of treatment. The assessment of prognostic variables only showed a significant association between longer duration of chemotherapy and risk of infection (Student t: 3. 08; p = 0. 002), and between infection and the risk of removal of the device (chi2: 17. 2, p = 0. 00004). **Conclusions.** The registered frequency of PAC infection and thrombosis in our patients is similar to that described in other clinical settings (i. e, non myeloma patients), as well as the aetiology of infections, being CNS the most frequently implied. Antibiotic lock was remarkably efficacious to avoid removal of PAC devices. Consequently, PAC devices appear to be safe in the chemotherapy of MM patients, without involving a higher risk of infection or thrombosis than that observed in different clinical settings, despite the usually longer time of use of the devices in these patients.

1545

THE IMPACT OF THE NOVEL THERAPIES BEFORE AND AFTER FRONTLINE AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background. High-dose therapy followed by autologous stem-cell transplantation (ASCT) is the standard of care for frontline therapy in younger patients with multiple myeloma (MM). During the past 10 years, novel agents such as thalidomide and bortezomib have been widely incorporated with improving the response rates. However, it is not clear whether such therapies are superior as induction treatment prior to transplantation or whether they may improve outcome as post-transplant maintenance therapy. **Aims.** To evaluate the association of survival outcomes in patients who underwent ASCT with or without the use of novel agents. **Methods.** We performed a retrospective analysis of 171 patients with MM who underwent ASCT within one year of diagnosis at our institute between 1999 and 2010, as part of first line therapy. **Results.** Their median age was 53 years (range, 34-65 years) and 90 (52. 6%) were male. Novel agents were administered to each patient as follows: before ASCT alone (n=41, 24. 0%), after ASCT alone (n=15, 8. 8%) and before and after the transplantation procedure (n=69, 40. 4%). Forty six (26. 9%) patients did not receive novel agents before and after ASCT. At a median follow-up of 43. 2 months, the 3-year overall survival (OS) and progression-free survival (PFS) rates were 66. 7% and 42. 0%, respectively. OS rates of the patients who received novel agents before and after ASCT were significantly increased compared to those with novel agents as induction therapy alone (P=0. 016) or those without novel agents (P=0. 020). In contrast, there was no difference between the patients who received post-transplant maintenance treatments regardless of use of the induction novel agents. Multivariate analysis showed that factors independently predictive of OS included achievement of CR after ASCT (P=0. 003), International Staging System (P=0. 026) and maintenance treatment with novel agents after ASCT (P=0. 034). **Conclusions.** These findings suggest that the integration of novel agents into the transplantation sequence is highly beneficial to patients with MM. In particular, administration of these agents after ASCT seems to more effectively improve OS.

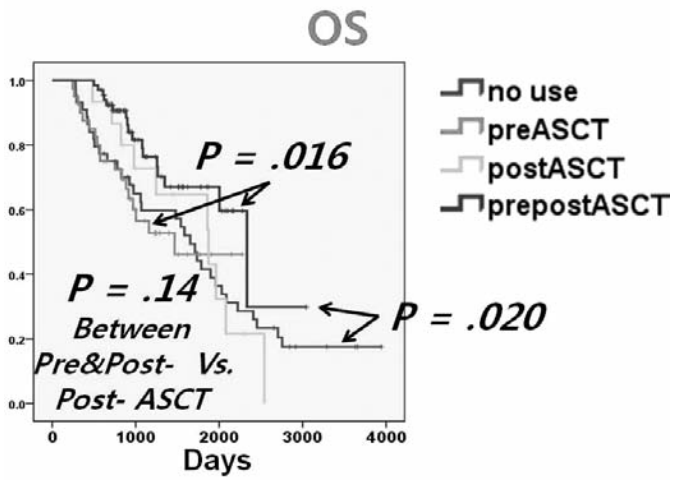

Figure 1.

1546

A NEW INDUCTION COMBINATION THERAPY (THALDODEX) FOR MULTIPLE MYELOMA. PRELIMINARY Results OF A PHASE II STUDY

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Background. treatment for multiple myeloma is dramatically changed over the past 5 years. Before the advent of the new drugs, anthracycline containing regimens were considered the gold standard in induction. **Aims.** to investigate the efficacy and safety of an antracycline based treatment combining liposomal doxorubicin, Myocet, to steroids, Dexamethasone, and Thalidomide (ThalDoDex). **Methods.** from June 2007 to January 2012, ThalDoDex combination was delivered to 38 previously untreated multiple myeloma patients in preparation to autologous stem cell transplantation (ASCT). **Results.** median age was 56 years (range 42-72). Treatment schedule was as follows: Thalidomide 100 mg/day for 14 days then 200mg/day until the end of induction; Dexamethasone 40 mg days 1à4; Liposomal Antracycline (*MYOCET*) 50 mg/sqm day 1 for 4 cycles at 4 weekly intervals. LMWH 100UI/kg/day was added to all patients as DTV prophylaxis. The response criteria were defined according to Bladè et al. The population presented as follows: 10 patients were stage IIA and 24 patients stage IIIA and 4 IIIB; 22, 12 and 4 patients were ISS I, II, III respectively. Monoclonal immunoglobulin subtypes were as follows: IgG in 21 patients, IgA in 7 patients, IgD in 1 patient, light chains in 7 patients, plasma cell leukemia in 1 patient and no secretory multiple myeloma in the last one. Thirty-five cases are evaluable for response and the overall response rate (CR, nCR, VGPR, PR) was 74%: CR + nCR + VGPR 54%. Thirty-one of 35 patients underwent ASCT and 50% of these maintained a response ≥ VGPR after the procedure with a median follow up of 24. 9 months (range 4-51). None of the patients who achieved a CR after TALDODEX regimen have then experienced a disease progression after transplant. **Conclusions.** our experience suggests that ThalDoDex is effective and safe in newly diagnosed multiple myeloma patients undergoing autologous stem cell transplant. Although the relatively short follow up, none of patients achieving a CR after TALDODEX progressed: our data confirm the relevance of CR before ASCT.

1547

INFECTION COMPLICATIONS IN AN UNSELECTED COHORT OF PATIENTS WITH MULTIPLE MYELOMA TREATED WITH LENALIDOMIDE COMBINATIONS

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Background. IMiDs exert various effects on the immune system, altering +cytokine production, regulating T cell costimulation and enhancing NK cell cytotoxicity. Particularly, IMiDs inhibit TNF□, playing an important role in immune response against bacterial and virus infections. Moreover, lenalidomide causes myelosuppression, mainly neutropenia that is an important risk factor for infections. **Aims and Methods.** We assessed the incidence, type and major factors affecting infections in 127 patients with MM receiving lenalidomide-based regimens. Median age was 67 years (40-88) and 53. 5% were older than 65 years. Fiftyfour patients (42. 5%) had newly diagnosed MM, whereas the remaining 73 (57. 5%) had relapsed/refractory disease. Among this group, 67% of patients received more than one line of therapy and 26% underwent APBSCT. ISS stage 2-3 and renal failure were recognized in 54. 5% and 8. 5% of patients, respectively; 15% had a ECOG PS □ 2. Eighty patients (63%) received lenalidomide plus dexamethasone and 47 (37%) lenalidomide combined with steroids and chemotherapy. Median number of lenalidomide courses was 6 (1-28) and 44% of pts developed neutropenia (26% grade 3, 8. 7 % grade 4). Nearly all patients (95%) received both trimethoprim-sulfamethoxazole as infections prophylaxis and granulocytecolony stimulating factor according to guidelines. **Results.** Twenty-six patients (20. 5%) developed infections resulting of grade 1-2 in 8 patients (6%) and 3-4 in the remaining 18 (14%). Two deaths (1. 5%) due to infections were observed. Type of infections were: pneumonia in 15 (58%), upper respiratory tract in 3 (11. 5%), FUO in 3 (11. 5%), septic shock by gram-negative microorganisms in 2 (8%), cholecystitis in 2 (8%) and VZV in one (4%). Risk of grade 3-5 infection was 16% at 12 months; 62. 5%, 69% and 94% of infections occurred at 3, 4 and 6 months, respectively. Among all the parameters evaluated in univariate and multivariate analysis, only ISS resulted as factor affecting severe infection development. Particularly, the risk of grade 3-5 infections at 6 months was 18% in patients with ISS 2-3, compared with 6% in those with ISS 1 (p=0. 034). A trend for a longer PFS in patients without infection (median PFS = 8 vs 16 months in patients with or without infections, respectively, p=0. 064) was documented; however, OS of patients developing infections was significantly shorter, compared to patients who did not develop infections (median OS=26 vs 33 months; p=0. 001). Multivariate analysis adjusted for age, PS, ISS, renal function and therapy with 2 or 3 drugs showed that infections significantly affected OS (HR=3. 2; 95%CI=1. 5-6. 7; p=0. 002). **Summary and Conlusions.** In clinical practice, infections represent a frequent complication in patients with MM receiving lenalidomide-based regimens and respiratory infections accounted for a large majority (nearly 70%). Nearly all patients who developed infections during the first 6 months of therapy and those with higher tumour burden have been found to be at higher risk. In conclusion, a broader antibiotic prophylaxis should be taken into consideration (ie TMP-SFZ plus fluoroquinolones) to prevent severe infections and ameliorate final outcome of MM patients at high risk (ISS 2-3) treated with lenalidomide combinations.

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A RETROSPECTIVE ANALYSIS OF THE CLINICAL RESPONSE RATE AND RENAL REVERSIBILITY OF THALIDOMIDE BASED REGIMES IN PATIENTS WITH MULTIPLE MYELOMA AND ASSOCIATED RENAL IMPAIRMENT

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Background. Patients with multiple myeloma (MM) frequently have renal impairment (RI), a significant complication with adverse prognostic significance. Rap-

id reduction of free light chains is critical in reversing RI secondary to cast nephropathy. Thalidomide is the first immunomodulatory drug with proven activity in MM and does not require dose reduction in RI. However, there is limited experience with thalidomide in patients with RI, and the current treatment of choice is bortezomib with high dose dexamethasone. **Aims.** To assess the safety and efficacy of thalidomide, cyclophosphamide and dexamethasone (CTD) or thalidomide with dexamethasone (Thal-Dex) in patients with MM and RI, and to evaluate RI reversibility. **Methods.** This is an observational retrospective study of patients with MM and RI, treated with thalidomide containing regimens. Patients with MM were identified using pharmacy prescriptions for thalidomide (2006 to 2011). Patients with concomitant RI were subsequently identified from laboratory investigations at the time of treatment initiation. **Results.** We identified 23 patients, with moderate to severe RI (Table 1) treated with CTD (12), CTDa (3) and Thal-Dex (8). The median number of cycles was 5. Two patients proceeded to autologous stem cell transplantation. The overall response rate (ORR) in all patients with RI (at least partial response, PR) was 74% (Complete Response, CR = 2; Very Good Partial Response, VGPR = 10; PR = 2) excluding four patients lost to follow up. The overall renal response rate (ORRR) (at least Minimal Response, MR) was 65% (CR= 7, PR= 2, MR= 6) with four of five patients becoming dialysis independent. In the moderate RI group the ORR was 77% (CR = 2, VGPR=4, PR=1) and the ORRR was 55%, (CR = 5). In the severe RI group, the ORR was 70% with four patients lost to follow up (VGPR = 6, PR = 1) and the ORRR was 71% (CR = 2, PR = 2, MR = 6). The median progression-free survival was 549 days. After a median follow-up of 877 days, three patients had died. Nine patients had renal biopsies; eight showed cast nephropathy and one, established tubular atrophy with acute tubular damage. Four other patients had associated hypercalcamia (range 2. 79-3. 23mmol/l). In one case, correction of hypercalcaemia led to improvement of RI. Three patients had pre-existing chronic kidney disease, and there was no subsequent improvement of RI. With regards to toxicity, hyperkalaemia was noted in one patient with severe RI. Thalidomide was withheld for five days, and hyperkalaemia did not recur after reintroduction. Two patients had grade II neuropathy (WHO classification). In one patient, grade III neuropathy required thalidomide withdrawal. **Summary and Conclusions.** Our results demonstrate that CTD and Thal/Dex are well tolerated in MM patients with RI. The haematological and renal response rates are comparable to those reported with bortezomib. Patients with severe RI had excellent outcomes. Our data, along with the current literature demonstrate that thalidomide based regimes in RI are both well tolerated and effective. This data would support further clinical trials in myeloma comparing thalidomide with bortezomib containing regimes in patients with associated RI.

Table 1. Patient's features at baseline.

No of patients	23
Male/Female	10/13
Median Age	70
Isotype: IgG κ/ IgG λ/ IgA κ/ IgA λ κ Light chain/ λ Light chain	7/ 2/ 4/1 6/ 3
Stage : I/ III	1/ 22
Previous therapy: 0/ 1	19/ 4*
Response to previous therapy: Yes/ No	1/ 3
GFR (ml/min): 30- 50 (Moderate RI) <30 (Severe RI)	9 14
**Cyclophospamide/ Dexamethasone (2),* Bortezomib/ Dexamethasone (1) Melphalan/Dexamethasone (1).	

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IMMUNOGLOBULIN'S SPECIFIC HEAVY/LIGHT CHAINS PAIRS ABNORMALITIES IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMIDED SIGNIFICANCE

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Introduction. Monoclonal gammopathy of undetermined significance (MGUS) is a condition in which a paraprotein is found in the blood during standard laboratory tests. It resembles multiple myeloma and similar diseases, but the levels of antibody (immunoglobulins) are lower, the number of plasma cells (white blood cells that secrete antibodies) in the bone marrow is lower, it has no symptoms or problems, and no treatment is indicated. However, multiple myeloma develops at the rate of about 1-2% a year, so clinicias recommend monitoring it yearly. Recently, the international myeloma working groups (IMWG) have established guidelines in order to stratify the risk of progression to multiple myeloma (MM). In this model, the amount and type of monoclonal protein together with ratio of serum free light chains (sFLC) are risk factors for progression. Recently, new assays that allow the identification and quantification of specific immunoglobulin heavy/light chains pairs (IgGk, IgGL; IgAk, IgAL; IgMk, IgML) have been developed. **Aims.** The aim of the present work is to study if MGUS patients also present specific heavy/light chains (HLC) alterations. **Materials and Methods.** A group of MGUS patients (N=59) was risk stratified according to the IMWG guidelines. All the patients had the serum M-spike quantified by serum protein electrophoresis, identified by serum immunofixation(sIFE) and serum free light chains (FreeliteTM, sFLC) were quantified by nephelometry. Immunoglobulin specific heavy/light chains pairs (IgGk, IgGL; IgAk, IgAL; IgMk, IgML) were also requested for all the study participants. The inclusion risk factors (IMWG) were: M-spike > 1,5 g/dL; type of immunoglobulin (Ig) by sIFE different from IgG; sFLC ratio < 0,26(L) or > 1,65(k)). All the patients were classified as High, High- intermediate, low-intermediate and low risk of progression according to the number of altered risk factors (3, 2, 1 or 0 respectively). The correlation between M-spike and monoclonal HLC pair was also established. **Results.** Among the selected MGUS population (2 biclonal; 32 IgG; 12 IgA; 13 IgM), 41% of the patients presented a low-intermediate risk of progression, 26 % had a low risk and a 33% presented a high-intermediate risk for progression. HLC ratios were altered in all except 2 IgG low risk patients. 30/32 IgG(94%); 11/12 IgA(92%); 12/12 IgM (100%) presented increased the monoclonal HLC. 15/32 IgG (47%); 9/12 IgA (75%) and 7/11(64%) MGUS pts presented the uninvolved HLC isotype immunosupressed. When the M-spike quantification was compared vs quantification of the monoclonal HLC pair, we found a moderate correlation for IgGk and IgAL (r2=0,51; r2=0,69), and very good correlations for IgGL, IgAk, IgMk, IgML (r2=0,81; r2=0,83; r2=0,80; r2=0,93). **Conclusions.** Due to the high sensitivity of the HLC ratio to indicate monoclonality, HLC assays could be of great utility to quantify monoclonal components, special those hidden by other proteins in patients with an IgA or IgM M-spike. Larger studies are need to determine the value of HLC assays as risk factor for progression however, immunosupresion of the uninvolved monoclonal isotype is seen frequently in MGUS patients and could play a role as progression marker.

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CONTEMPORARY INTRAVENOUS INJECTION OF BORTEZOMIB, MELPHALAN AND DEXAMETHASONE IN REFRACTORY AND RELAPSED MULTIPLE MYELOMA

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Background. Combination of Melphalan and Bortezomib is highly active in multiple myeloma (MM). However, several clinical pharmacokinetic studies have shown that melphalan has a short half-life in the plasma, and its absorption from the gastrointestinal tract is extremely variable. Considering the frequency of bortezomib-related gastrointestinal toxicity, it is reasonable to hypothesize that bortezomib could also hamper the melphalan absorption thus reducing its activity. **Aims.** To ensure that all the drugs were present in the plasma at the same time and at the most effective concentrations, we designed a protocol with the contemporary intra-venous (IV) administration of melphalan, bortezomib and dexamethasone (BMD). **Methods.** Fifty previously treated (median 2 previous lines) myeloma patients (33 relapsed and 17 refractory) were evaluated. Nine patients (18%) had already received bortezomib alone or in combination, and 35 patients (70%) had already received melphalan (17 at high dose as conditioning regimen for autologous stem cell transplantation and 18 as part of a standard melphalan- prednisone regimen). Bortezomib 1. 3mg/m^2 and melphalan 5 mg/m^2 were given IV contemporary to dexamethasone 40 mg in accord to bortezomib schedule 1,4,8,11 (*base*) or 1,8,15,22 (*weekly*), recycling after 14 days from the last dose; dexamethasone was administered orally the day after each injection, for a median of 4 cycles (1-6). **Results.** The overall response rate (ORR) was 62%, with high rate of complete responses (CR, 26%). After a median follow up of 24. 5 months, median PFS and OS were respectively 21. 6 and 33. 8 months without significant differ-

ences between *weekly* schedule (respectively 16. 2 and 23. 4 months) and *base* schedule (respectively 23. 6 and 34. 6 months). Independently from the adopted schedule, achieving at least partial remission (PR) improved PFS (35. 2 vs 9 months) and OS (unreached median vs 18 months). Side effects were predictable and manageable, with prominent haematological grade 3-4 toxicity, including thrombocytopenia (52%), anemia (24%) and neutropenia (18%). Gastrointestinal disturbances (27%) and peripheral neuropathy (13%) never exceeded grade 3. *Weekly* schedule was better tolerated than *base* schedule, and it did not required dosage reduction. **Conclusion.** BMD is an effective salvage regimen in relapsed/refractory myeloma patients with manageable and acceptable toxicity, especially when used in accord to the *weekly* schedule.

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IMPACT OF BORTEZOMIB IN THE SURVIVAL OF PATIENTS WITH REFRACTORY OR RELAPSED MULTIPLE MYELOMA WITH RENAL FAILURE. EXPERIENCE OF A SINGLE CENTRE.

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Background. The introduction of Bortezomib (V) and the new biological treatments in recent years has improved overall survival (OS) of the patient with multiple myeloma (MM). There is insufficient information on the effect of these new therapies in patients with renal insufficiency (RI). **Aims.** We report our experience in patients with refractory or relapsed MM treated with V and analyze survival in groups with and without RI. **Methods.** 62 patients with Multiple Myeloma that had relapsed or were refractory to conventional chemotherapy were treated with V as a single drug or in combination (dexamethasone or doxorubicin). Demographic and clinical features, frequency and severity of RI (defined as Cr plasma ≥1, 5 mg/dl, excluded functional factors), acute hemodialysis (AHD) and chronic hemodialysis (CHD) have been assessed. Kidney function,paraprotein and Bence Jones protein were registered baseline, after 2 cycles of V (pre3) and at the end of the treatment (post). We analyzed overall survival by Kaplan Meier from diagnosis and since the beginning of V in both groups, with and without RI. Markers of development of RI have been identified by binary logistic regression. **Results.** Age at presentation was 65,58, SD: 11. 5 (37, 7-82, 1). 58. 8% were men. RI in 24/62 p (39%). Cr pre V: 3. 86 mg/dL, SD: 2,17(1,5-12,4), Bence Jones pre V: 147,8 mg/dL, SD: 110,6 (2-600) and quantification of monoclonal paraprotein preV: 2. 57 mg/dL, SD: 1. 9. Cr pre3: 3. 04 mg/dL, SD: 2. 4 (NS) and Cr post: 2. 69 mg/dL SD: 1. 4 (P = 0, 001). Paraprotein pre3: 1. 3 mg/dL, SD: 1. 7 (p = 0, 009) and post: 0. 54 (p = 0, 01). Bence Jones pre3: 62. 4 mg/dL, SD: 93,2 (P = 0, 02) and post: 71. 3 mg / dL, (NS). Associated hypercalcemia in 6/24 p (25%). 5/24 p (20. 8%) need AHD, with recovery of 3 patients and finally CHD in 6/62 p (9%). 50 % of women in RI group versus 37. 5% in the group without RI (p = 0, 04). Binary logistic regression analysis identifies age, sex and cuantification of light chain excretion at the diagnosis as predictors of RI. Overall survival of patients with RI was 1. 78 years (CI 95%: 1, 25-2, 3) versus 2. 02 years in those patients without RI (CI 95%: 1. 5-2. 5) NS. Patients who require AHD or CHD had worse survival than those patients with RI that do not require AHD/CHD, though this difference was not statistically significant. AHD: 0. 8 years (CI 95%: 0-1, 95) versus 3,028 years (CI 95%: 2, 4-3, 6) and CHD: 1. 1 years (CI 95%: 0-2, 3) vs. 3. 31 years (CI 95%: 2, 8-3, 8). **Conclusions.** In our experience Bortezomib is effective in the control of acute renal impairment in patients with refractory or relapsed multiple myeloma. Age, sex and ligeruria are identified as predictors of development of renal failure. We did not find differences in survival among the group with renal failure and the group without renal failure.

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EFFICACY OF INDUCTION TREATMENT WITH CYCLOPHOSPHAMIDE, THALIDOMIDE AND DEXAMETHASONE (CTD) CHEMOTHERAPY IN NEWLY DIAGNOSED MYELOMA PATIENTS ELIGIBLE FOR THE AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. A novel agents like thalidomide, bortezomib and lenalidomide as optimal partners to standard and high-dose therapy followed by the autologous stem cell transplantation (ASCT), dramatically changed the course and prognosis of patients with multiple myeloma (MM). **Aims.** The aim of study was to analyze efficacy of the induction treatment with CTD chemotherapy in newly diagnosed MM patients (pts) eligible for the autoSCT. **Patients and Methods.** We have analyzed 57 newly diagnosed MM pts (32 male/ 25 female, mean age 55 yrs, range 40-65) treated with induction CTD chemotherapy. IgG myeloma was diagnosed in 31pts, IgA in 12pts, light chains in 11pts and non-secretory in 3pts. According to the clinical stage (CS, Durie-Salmon), patients were distributed as follows: II 20pts; III 37pts. Regarding ISS score, the group included: ISS1 10pts; ISS2 25pts; ISS3 22pts. Renal impairment was present in 5pts. Induction treatment according to the CTD regimen (Cyclophosphamide 500mg p. o. 1, 8, 15. day, Thalidomide 100mg/day p. o, Dexamethasone 40mg/day i. v. 1-4, 12-15. day of three-week cycle, mean no. 4 cycles, range 3-6 cycles) was applied in all patients. Stem cell mobilization was performed with combination of CAD chemotherapy and G-CSF, in 47/57 (81%) patients who achieved CR/VGPR/PR (IMWG criteria). Adequate number of CD34+ cells (>5x10e6/kg BW) was obtained in 40/47pts (85%). In 8/40pts (20%), mobilization and apheresis were performed twice in order to collect adequate number of CD34+ cells. High-dose therapy (HDT) with Melphalan 200mg/m² and ASCT were performed 4-8 weeks after mobilization in 35/47pts (74%), followed with Thalidomide maintenance (Thal 100mg/day, median duration 16m, range 6-24m) in patients with CR/VGPR/PR. Routine thromboprophylaxis was applied in all pts. **Results.** CTD induction treatment resulted with CR in 7/57pts (12,3%), and VGPR/PR in 40/57pts (70,2%). Further improvement of the response was obtained after HDT and autoSCT (CR 9/35pts, 25,7%; VGPR/PR 21/35pts, 60%). Median follow-up was 20m (6-36m). The 3-yrs probability of event-free, relapse-free and overall survival of 35/57pts treated with CTD+HDT+Thal was as follows: EFS 48%, RFS 60%, and OS 87%. The main reason for thalidomide discontinuation was peripheral neuropathy recorded in 21/35pts (60%) with occurrence of grade 3-4 toxicity in 6/35pts (17%). Thrombosis was not a risk in this setting. **Conclusions.** CTD chemotherapy represents highly effective and relatively safe induction in MM patients eligible for the autoSCT. HDT followed by Thalidomide maintenance improves quality and duration of the response, as well as overall survival, mainly due to the better quality of response and further reduction of tumor mass after HDT. However, recommended duration of such treatment is to be defined predominantly due to the limitations caused by the toxic effects.

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SINGLE-AGENT CARFILZOMIB VERSUS A BEST SUPPORTIVE CARE REGIMEN IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA: FOCUS (PX-171-011), A RANDOMIZED, OPEN-LABEL, PHASE 3 STUDY

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Background. The proteasome inhibitor carfilzomib has demonstrated single-agent activity in phase 2 studies in patients with relapsed and refractory multiple myeloma (R/R MM). In PX-171-003-A1"'a single-arm study of carfilzomib monotherapy in heavily pretreated refractory patients"'37% of patients achieved > minimal response (MR), median time to progression was 3. 9 months, and median overall survival (OS) was 15. 5 months. Carfilzomib is currently under review by the US Food and Drug Administration for approval to treat R/R MM. **Aims.** Herein we describe the trial design and rationale for a phase 3 randomized study, FOCUS (CarFilzOmib for AdvanCed Refractory MUltiple Myeloma European Study), being conducted to compare OS after treatment with single-agent carfilzomib to a "best supportive care"(BSC) regimen (low-dose glucocorticoids with optional cyclophosphamide, plus comfort and palliative care). **Methods.** Patients must have received ≥3 prior regimens including bortezomib treatment (defined as ≥4 cycles at full dose as tolerated). Patients must have been responsive to ≥1 line of therapy and be either nonresponsive (≤ stable disease) or refractory to their most recent therapy. Eligible patients are randomized 1:1 (stratified by number of previous therapies and geographical region) to carfilzomib or BSC regimen. Target enrollment is 302 patients. Treatment schemes are:Treatment will continue until disease progression or unacceptable toxicity. Following confirmation of disease progression or discontinuation from study treatment, all patients will enter long-term follow-up for survival. Crossover is not allowed upon progression. The primary endpoint is OS, and secondary endpoints include progression-free survival, overall response rate, clinical benefit rate, disease control rate, duration of response, and safety. Disease assessments will be determined by study investigators and an independent review committee according to the International Myeloma Working Group Uniform Response Criteria (with MR per European Blood and Marrow Transplantation Group criteria). FOCUS began accruing patients in September 2010, and 141 patients are enrolled as of the end of January 2012. **Conclusions.** FOCUS will provide more rigorous data for carfilzomib, as this is the first carfilzomib study with OS as the primary endpoint and it will not be confounded by crossover. The larger study group will also provide more robust secondary response and safety results that will add to the data set from prior phase 2 studies. This phase 3 study of carfilzomib monotherapy for patients with R/R MM

will provide important information necessary to facilitate regulatory approvals around the world and to expand treatment options to meet the unmet need of these patients with advanced disease.

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FACTORS ASSOCIATED WITH INFECTIONS IN HOSPITALIZED PATIENTS WITH MULTIPLE MYELOMA: SINGLE CENTER EXPERIENCE

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Background. The disease-related immunological defects, as well as therapy-related immunosupression, lead to increased risk for infective complications which are a major cause of morbidity and mortality in patient with multiple myeloma. Several risk factors increase susceptibility to infections and their understanding is crucial in order to fight against this complications. **Aims.** 1) to establish factors associated with infections in hospitalized myeloma patients at the Department of Hematology, Clinical Hospital Center Rijeka in a three year period 2) to see the incidence and the characteristics of infections in these patients. **Methods**. Retrogradely, from the hospital medical documentation, we processed 241 cases of patients with multiple myeloma who were hospitalized in our Department from January 2008 to December 2010. This study did not include patients who were treated with high dosage therapy and stem cell transplantation. The factors which could induce the infections, such as sex, age, stage of disease, International Staging System, renal failure, imunoparesis, neutropenia, ECOG Preformance Status, serum ferritin, different catheters, were compared and statistically analyzed in the group of patients with and without infections. The frequency and the basic characteristics of the identified infections (microbial agents, site, time of occurrence and outcome of infection, as well as the type of antitumor therapy at the time of infection) were also determined. **Results**. Infections were identified in 44 out of 241 (18, 25 %) cases of hospitalized patients. We established that the factors associated with the more frequent infections were: female sex (p=0,001), clinical stage of disease IIIB (p=0,007), increased serum creatinine levels (p=0,036), neutropenia (p=0,009), poor general condition (p<0,001), high serum ferritin level (p=0,001) and presence of catheters (p<0,001). The most common pathogens found were P. aeruginosa in 9 cases (16,6%), E. coli in 5 (9,25%) as well as Staph. epidermidis in 5 (9,25%) and Strept. foecalis in 5 cases (9,25%). The frequency of Gram-positive and Gram-negative pathogens was similar. In 17 (38,63%) cases we did not manage to isolate the agent. The most common sites of infections were urinary system in 16 cases (36,4%) and blood in 11 cases (25%). Infections generally occured in the later stage of the disease (relapsed/refractory patients) with the highest frequency in patients treated with bortezomib (30,2% of all cases with infections were treated with bortezomib-based therapy as third line of therapy). Fatal outcome occurred in 4 out of 44 cases (9. 09 %). **Conclusions.** This study established female sex, IIIB clinical stage, increased serum creatinine level, neutropenia, poor general condition, high serum ferritin and presence of catheters as possible risk factors for infections in hospitalized myeloma patients which can be contribution for better understanding and prevention of infections in myeloma patients.

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POEMS SYNDROME TREATED WITH MELPHALAN HIGH-DOSE THERAPY AND AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION: A SINGLE INSTITUTION EXPERIENCE

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Background. The acronyme POEMS syndrome stands for a rare multi-system disorder comprised of polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes. Treatment of POEMS may include alkylator-based therapies, and/or a combination of dexamethasone either with anthracycline or melphalan (MDex). Some single centers reported efficacy of melphalan high-dose therapy (HDT) in combination with autologous blood stem cell transplantation (ABSCT) in small patient cohorts with progressive POEMS syndrome. **Aims.** This retrospective analysis aimed to evolve clinical end points including overall survival and clinical outcome of 5 patients with POEMS syndrome treated with melphalan HDT and subsequent ABSCT in our medical center. **Methods.** From 1996 until 2011, 5 patients were diagnosed with POEMS syndrome at the University Hospital of Muenster, Germany, based on the major and minor criteria for POEMS syndrome [Dispenzieri A, *Blood* 2007]. Median age at diagnosis was 44 years (range: 32 - 58). All but one patient had received conventional treatment regimens including anthracycline/dexamethasone or MDex before HDT. One patient was immediately prepared for HDT/ABSCT due to massive splenomegaly and high-grade polyneuropathy at the time of POEMS diagnosis. Between January 2002 and March 2011, all patients developed progressive disease, and therefore underwent melphalan HDT with subsequent ABSCT after giving their written consent. Peripheral blood stem cell collection was performed either following cytotoxic chemotherapy or steady state using stimulation with granulocyte colony-stimulating factor (G-CSF, 5 µg/kg/d s. c.). For cytotoxic mobilization, IEV chemotherapy [intravenous ifosfamide (2500 mg/m^2 day 1-3), epirubicin (100 mg/m^2 day 1) and etoposide (150 mg/m^2 day 1-3)], followed by G-CSF (5 µg/kg/d s. c.) was used. For HDT regimen melphalan 200 mg/m^2 was applied. The median number of CD34-positive cells transfused was 3. 93x10^6/kg (range 2. 1-5. 44). All patients were provided with prophylactic antibiotics and G-CSF (5 µg/kg/d s. c.), which was daily administered starting 24 hours after ABSCT until regeneration of peripheral neutrophils. Hematologic responses were defined according to the *International Myeloma Working Group criteria* [Rajkumar S *et al., Blood* 2011] and conferring to Dispenzieri [*Blood*, 2011]. VEGF testing was performed at the IMMUMED Laboratory, Munich, Germany. **Results.** After a median follow-up of 18 months (range: 11 - 120), all patients are alive and achieved clinical improvement especially of their neurological deficits. In two cases, HDT followed by ABSCT resulted in a complete hematologic response; in the additional three cases, partial responses (PR) were achieved including one very good hematologic PR. Only one patient with initial PR developed progressive disease nearly 2. 5 years after transplantation. Consequently, a second HDT with ABSCT was successfully applied resulting in clinical improvement and hematologic PR. High initial serum VEGF levels were available in two patients and were monitored after HDT and ABSCT. In accordance with literature, correlation was noted between the status of the disease and the corresponding VEGF level in both patients after melphalan HDT and ABSCT. Overall, no severe transplantation-associated complications such as engraftment syndrome or peri- or post-transplant death were noted. **Conclusions.** Melphalan HDT followed by ABSCT is an effective first line treatment for POEMS patients with progressing symptoms.

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BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY IN MULTIPLE MYELOMA PATIENTS

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Bortezomib, a class A proteasome inhibitor, influences various cell signaling pathways and inhibits nuclear factor κB (NF κB). Bortezomib-induced peripheral neuropathy (BIPN) is a common adverse effect. We studied the incidence, risk factors and outcome of BIPN in 93 multiple myeloma (MM) patients. The study group consisted of 55 male and 38 female patients of median age 60 years (range 32-81) who received a median of 4 cycles (range 1-9) of bortezomib. Sixty-eight (73%) of them had advanced stage (refractory/relapsed) disease and 25 (27%) early stage disease. Among the latter, 8 received bortezomib as first-line treatment and 17 before HCT. Disease stage at diagnosis was III in 53 (56%) patients; paraprotein was IgG in 52 (56%), IgA in 23 (25%), IgD in 1 (1%), light chain only in 10 (11%), non secreting in 7 (7%). Eighteen (19%) of them had renal impairment at diagnosis with creatinine levels higher than 2 mg/dl. Bortezomib was co-administered with dexamethasone in 38 patients, anthracycline (PAD regimen) in 16, melphalan with corticosteroids (VMP regimen) in 8. BIPN was noticed in 55/93 (59%) patients after a median of 3 (range 1-7) cycles. In the majority of patients (47/55, 85%) neuropathy was grade 1 or 2. It was diagnosed mostly clinically, whereas 14 patents underwent electrophysiology testing. In all patients BIPN was sensory, in 29/55(53%) neuropathetic pain/nocturnal causalgia coexisted and only in 8/55(14%) motor neuropathy was observed. Four patients presented with bowel autonomic neuropathy, ie mild paralytic ileus. Patients treated earlier in the disease course (0-2 previous lines of treatment) had a similar BIPN incidence with heavily pretreated MM patients (53% and 60% respectively, p=ns). Twenty two patients (40%) needed treatment for BIPN such as pregabalin. Dosage or administration interval adjustment was necessary in 26/55 (47%) patients. Treatment was discontinued in 10/55 (18%) because of BIPN severity or patient denial. Bortezomib retreatment in 6 patients did not produce any additional neurotoxicity. BIPN recovered totally in 43/55 (78%) or gradually ameliorated in 12/55 (22%)

patients. In multivariate analysis, incidence and grade of BIPN did not correlate to factors as age, disease stage, renal impairment or other treatment-related toxicities (e. g. neutropenia, thrombocytopenia). However, there was a significant correlation to the cycles of bortezomib administration and the response to therapy. Conclusively BIPN is a common adverse effect of bortezomib. In most patients it is mild and self restricting. Timely diagnosis and application of dosage adjustment guidelines are important, as there is no specific or highly effective treatment.

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FAMILIAL MULTIPLE MYELOMA: DESCRIPTION OF FIVE FAMILIES WITH TWO FIRST-DEGREE RELATIVES DIAGNOSED WITH MM AND TWO FAMILIES WITH A THIRD CASE OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Introduction. The Familial Multiple Myeloma (fMM) frequency is 3. 2 per 1000 cases of Multiple Myeloma (MM), with an incidence of 0. 1 cases per million people per year, which ranks it as a very rare event. However, the risk of relatives developing MM is 1. 7 times and MGUS, 2. 9 times, greater than that of the general population. The finding of fMM may be due to environmental or hereditary cases with limiting factors described for husband and wife and monozygotic twins. **Case Studies.** Family1: The index case was a black man, aged 38, diagnosed with MM IgG kappa IIIA in 1998. In 2007, his brother, aged 59, was also diagnosed with MM IgG kappa IIIA. This family consists of ten siblings. During evaluation of the remaining eight brothers, with examination of protein electrophoresis and immunofixation serum and urine screening, we identified a case with a monoclonal peak less than 1. 5 g/dl and serum immunofixation monoclonal IgG kappa, characterizing an IgG Kappa MGUS case. In the two MM cases of this family, the same HLA profile - (A, B, C, DRB1, DQB1) was found. Family 2: The index case was a white woman, aged 44, diagnosed with MM IgG IIIA in October 2000. In 2009, his mother, aged 74, was diagnosed with MM IgA kappa IIIA. In this family with nine siblings, one died at two years old and others were investigated, identifying a monoclonal peak case with less than 1. 5 g / dl, serum immunofixation monoclonal IgA lambda, characterizing an MGUS IgA lambda case. Family 3: The index case was a white man, aged 67, diagnosed with MM IgG lambda IIIA in January 2008. His first cousin, white, aged 65, received a diagnosis of MM IgA kappa IIIA in June 2009. We performed an investigation of the brothers of the index case, who were normal. The second family was not investigated due to its limited access to our hospital. Family 4: The index case was a black man, aged 50, diagnosed with MM IgG kappa an ISS 1 in August 2007. His father, black, aged 82, received a diagnosis of MM IgA lambda IIIA in January 2010. The index case brothers are under investigation for the presence of gammopathy. Family 5: The index case was a white woman, aged 90, diagnosed with MM IgG kappa ISS1 in June 2004. His daughter, white, aged 64, was diagnosed with MM IgG kappa IIIA ISS3 in September 2011. She has a sister who is under investigation. **Conclusions.** The present study describes five families with more than one case of MM. In two of the five families, two MGUS cases were diagnosed. Moreover, in one family, the immunoglobulin subtype, as well as HLA, was identical. This is the first report of this finding in the literature. This study establishes a precedent for the diagnosis of familial MM, considered a risk factor for other members of the same family to develop some kind of gammopathy.

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SERUM IMMUNOGLOBULIN HEAVY/LIGHT CHAIN (HLC) AND FREE LIGHT CHAIN (FLC) CONCENTRATIONS AT DIAGNOSIS IN MULTIPLE MYELOMA (MM) AND IGM MALIGNANT LYMPHOMA (ML) PATIENTS WITH SURVIVAL EXCEEDING 10 YEARS

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FLC assay measures circulating unbound kappa and lambda light chains and is of diagnostic and prognostic value in plasma cell disorders. HLC is a novel antibody based assay that targets the unique junctional epitopes between the heavy chain and light chain constant regions of intact immunoglobulin (Ig) molecules. It separately measures in pairs the light chain types of each immunoglobulin class generating ratios of monoclonal Ig/ background polyclonal Ig concentrations (i. e. IgGκ/IgGλ, IgAκ/IgAλ and IgMκ/IgMλ). (Bradwell et al. Clin Chem 2009, 55: 1646). To assess the prognostic impact of these assays we analyzed presentation stored serum samples from 23 MM(14 F9M, median age 52 years, range 32-78, 7 IgGκ, 6 IgGλ, 8 IgAκ, 2 IgAλ; ISS stage I: 16, stage II 6, stage III 1) and 12 ML (7F5M, 9 IgMκ, 3 IgMλ) patients with survival exceeding 10 years (up to 26 years) and 43 (19 MM, 24 ML) patients with survival not exceeding 5 years. All patients were diagnosed and follow-up for many years in our Institute. Serum FLC and HLC (IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ) measurements were made on a Siemens BN™ II nephelometer using polyclonal antisera assays (Freelite™ and Hevylite™, Binding Site,Birmingham,UK). Serum protein electrophoresis and immunofixation were conducted on a SEBIA Hydrasys II apparatus. In all patients HLC and FLC ratios were abnormal. In patients with survival exceeding 10 years: median values (and range) of HLC ratios were as follow: in MM IgGκ 11. 8 (7. 9-58. 5), IgGλ 0. 09 (0. 06-0. 35), IgAκ 20. 9 (5. 1-79. 3), IgAλ 0. 09, 0. 16, in ML IgMκ 104. 0 (4. 2-2680), IgMλ 0. 04 (0. 01-0. 05); median values (and range) of FLC κ/λ ratio were: in MM IgGκ 8. 56 (2. 35-99. 80), IgGλ 0. 09 (0. 03-0. 12), IgAκ 4. 29 (1. 45-31300), IgAλ 0. 09, 0. 26, in ML IgMκ 11 (2. 0-28), IgMλ 0. 13 (0. 08-0. 61). In patients with survival not exceeding 5 years: median values (and range) of HLC ratios were as follow: in MM IgGκ 43. 29 (2. 14-73. 79), IgGλ 0. 01 (0. 01-0. 09), IgAκ 189. 0 (102-742), IgAλ 0. 02 (0. 02-0. 06), in ML IgMκ 280. 0 (9. 63-3610), IgMλ 0. 01 (0. 01-0. 05); median values (and range) of FLC κ/λ ratio were: in MM IgGκ. 189(5-7620), IgGλ 0. 03 (0. 001-0. 08), IgAλ 0. 01;0. 001, inML IgMκ 531, IgMλ 0. 01, 0. 06. When patients were stratified according to their survival time > 10 years or < 5 years serum HLC and FLC ratios at diagnosis were less abnormal in patients with survival excceding 10 years (p=0. 03). However in patients with survival > 10 years highly abnormal HLC ratio (<0. 022 or > 45) was found in3 MMpatients (13. 6%) and 7 ML patients (58%) and highly abnormal FLC ratio (< 0. 1 or > 30) was found in 5 MM patients (23. 8%) and in 1 ML patient (8%). Conclusions. Serum heavy /light chain and free light chain measurements at MM diagnosis, provide prognostic information despite that even in MM patients with survival exceeding 10 years in 15% at diagnosis serum HLC and FLC ratios may be highly abnormal.

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EFFECT OF CONTINUING LENALIDOMIDE TREATMENT ON SURVIVAL IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA A CLINICAL PRACTICE CHART REVIEW

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Background. Lenalidomide (LEN) in combination with dexamethasone has demonstrated significant improvements in progression free survival (PFS) and overall survival (OS) in patients with relapsed/refractory multiple myeloma who have received ≥ 1 prior therapy. San Miguel et al (Clinical Lymphoma,Myeloma&Leukemia, 2010) showed that in two large multicenter phase III trials (MM-009 and MM-010), continuing LEN plus dexamethasone after acheiving partial response leads to a significant survial advantage when controling for patient characteristics. In Slovakia, the approved length of LEN treatment according to local regulations is 8 cycles. Additional treatment could be reimbursed on exceptional basis. **Aims.** To conduct a clinical chart review (2/2010-7/2011) to collect data from relapsed/refractory multiple myeloma patients in everyday clinical practice and to evaluate the benefits of prolonged treatment (more than 8 cycles). **Methods.** At the time of this analysis, retrospective data were collected from 146 evaluable patients, 48. 63% male, median age 67 years. Patients had a median of 3lines of prior therapy and 41. 78% were starting second line treatment. Most patients (66,44%) were Durie-Salmon stage IIIA at time of starting lenalidomide treatment Patients received either LEN until disease progression (43 patients) or LEN until 8 cycles (103 patients). The majority of patients (98. 63%) received LEN in combination with a glucocorticoid, 17. 81% of those received dexamethasone,82,19% received other glucocorticoid. LEN dosage after 8 cycles was, 25 mg(4. 65%), 15mg(16. 28%) and 10 mg(79. 07%) . All patients received thromboprophylaxis. **Results.** After 8 cycles of LEN, 76. 72% acheived ≥PR(8. 91 % CR, 36. 99 % VGPR, 30. 82 % PR), 14% had stable disease, and 4. 79% had progressive disease, in 4,49% response not available . Median OS from the start of therapy was 37 months in LEN- continuing arm (95%CI, 30-44) and 32 months in LEN-8 month arm (95%CI, 21-43) . LEN continuing treatment did not result in significantly higher incidence of hematologic adverse events. Incidence of neutropenia (all grades) during the first 8 cycles was 35,62%, after 8th cycle was low, 4. 65%. One patient treated with 10 mg daily LEN continuing treatment experienced thrombosis. Two

patients treated with LEN continuing treatment experienced second primary malignancy (one lung cancer, one CLL). **Conclusion.** The results confirmed the survival benefit of prolonged LEN treatment. The results of this retrospective analysis provide the picture of unselected patients with RRMM in Slavokia The obtained data from this everyday clinical practice corresponds with the results of international clinical trials.

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CNS MULTIPLE MYELOMA - A MULTICENTRE EXPERIENCE OF A RARE MANIFESTATION

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Background. CNS involvement is a rare complication (1%) of systemic multiple myeloma (MM) and anecdotally appears to be becoming more prevalent in the era of novel therapy. CNS MM has a dismal prognosis with a reported median survival of 2 months and although there are reports of response to novel agents, data are inconsistent and there is no treatment consensus. **Aim.** To describe the clinical features, response to therapy and overall survival (OS) of patients with CNS involvement of systemic MM. **Methods.** Review of patient records from Jan 2000-May 2011 at 4 tertiary referral hospitals identified patients diagnosed with CNS MM defined by monoclonal plasma cells in CSF (meningeal myelomatosis) and/or radiological evidence of cerebral parenchymal plasmacytoma/leptomeningeal disease in the absence of an obvious alternative diagnosis in patients with systemic MM (biopsy not mandated for inclusion). A retrospective analysis of clinical and treatment data was performed. **Results.** Seventeen cases were identified., eleven with meningeal myelomatosis, three with leptomeningeal disease and three with intracranial plasmacytoma. At initial diagnosis, median age was 58 (41-70) years, 7 patients had ISS stage III disease, two presented with non-CNS extramedullary disease, and 3/10 evaluable had unfavourable cytogenetics. Lambda light chain restricted cases were predominant (11/17). Patients received a median of 3 therapies (1-4) prior to diagnosis of CNS MM, including autograft (n=16), allograft (n=2) and at least one of thalidomide (n = 14), lenalidomide (n = 4) or bortezomib (n =8). Median time to diagnosis of CNS MM was 36 months (1-114). Eight patients had concomitant progressive systemic disease,. Two patients demonstrated a shift in secretion from intact immunoglobulin to light chains ("light chain escape"). The most common clinical presentation was cranial nerve palsies (n=7) or headache (n=3). All patients received combinations of radiotherapy (RT) (n=12), intrathecal (IT) chemotherapy (n=8), systemic chemotherapy (n=3) and/or novel agents (bortezomib n = 2, thalidomide n = 5). The median OS from initial diagnosis was 47 (12-124) months but from time of diagnosis of CNS disease was only 4 (1-23) months. Those who received IT chemotherapy had superior OS [20 months vs. 2 months (p=0. 02)], but there was no improvement in survival for those who received RT (p=0. 91). CSF clearance was documented in the 2 of 11 patients who had positive CSF cytology and who received IT chemotherapy. Only 2 patients were alive at time of report (5 and 10 months post-diagnosis of CNS MM). Both received IT chemotherapy and bortezomib as part of their treatment. **Conclusions.** CNS MM is associated with a poor prognosis and currently has limited treatment options. Our cohort reflected many of the features and associations reported in the literature. Given the additional observation of light chain escape in two of our patients, we propose clinicians have a lower threshold for considering CNS involvement in patients with light chain escape who have suggestive symptoms. IT chemotherapy and bortezomib may be of benefit in selected cases but larger, prospective collaborative studies are required to test this observation. No conflicts of interest to disclose.

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MULTIPLE MYELOMA AND RENAL IMPAIRMENT: SURVIVAL TRENDS IN GREATER GLASGOW & CLYDE

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Background. New therapeutic agents shown to improve survival in multiple myeloma have become available over the past decade. Melphalan and prednisolone was the most commonly used chemotherapy in the 1990s. In 2000 thalidomide became available. In 2005 bortezomib and later lenalidomide became available as second line agents. Survival of patients with myeloma in Scotland has steadily improved over the last 30 years. Renal impairment is present in 20% at diagnosis and is associated with poor overall survival. **Aims.** We aimed to examine the baseline characteristics, treatment and survival trends of patients diagnosed with multiple myeloma and renal impairment presenting to renal services between 1990-2010. **Methods.** We identified 105 patients diagnosed with multiple myeloma from the Greater Glasgow & Clyde area with a presenting serum creatinine of greater than 130 micromol/litre using the electronic renal patient record (SERPR). Three cohorts were selected for analysis: 1990-99 (n=27), 2000-04 (n=29), 2005-10 (n=49). **Results.** The fact that the 1990-99 cohort had a lower mean age than the 2000-04 and 2005-10 cohorts (65. 5 v 70. 0 v 70. 0 years respectively; p=0. 13) and a lower proportion required long-term renal replacement therapy (RRT) from presentation (18. 5 v 44. 8 v 30. 6%; p=0. 1) suggests that more severe cases were less frequently diagnosed or referred to renal services in the earliest era. There was no statistically significant difference in overall actuarial survival between the 3 eras (median survival 1441, 488, 537 days respectively; p=0. 24 log rank). Analysis of patients not requiring long-term RRT from presentation showed no significant difference in overall survival or renal survival although there was a trend towards improved median overall survival between 2000-04 and 2005-10 eras (568 v 1022 days; p=0. 83). **Conclusions.** Multiple myeloma with renal failure remains a condition with poor outcome despite the availability of agents in the last decade that target the underlying disease process more effectively.

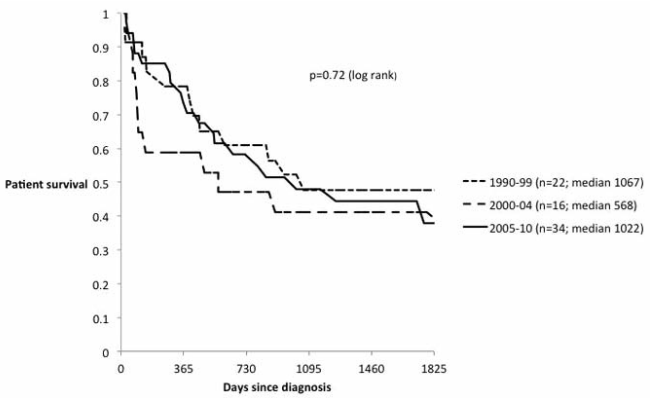

Figure 1. Patient survival for patients not requiring long-term dialysis at presentation analysed by era.

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AL AMYLOIDOSIS: A SINGLE CENTER EXPERIENCE

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Background. Light chain (AL) amyloidosis is a plasma cell dyscrasia characterized by the pathologic production of fibrillar proteins comprised of monoclonal light chains which deposit in tissues and cause organ dysfunction. Standard treatment approaches include high dose Melphalan (HDM) followed by autologous hematopoietic stem cell transplantation (SCT) or oral Melphalan with Dexamethasone (MD) or prednisone (MP). The use of novel agents (Thalidomide, Lenalidomide and Bortezomib) has shown efficacy and continues to be explored. **Aims.** To evaluate the prognostic factors and the outcome of a cohort of 29 AL amyloidosis patients treated with standard therapy and followed at a single center**. Methods.** We performed a retrospective study of 29 AL amyloidosis patients diagnosed and managed between September 2000 and July 2011 at our center. The diagnosis was made by Congo red staining of subcutaneous fat obtained by aspiration and/or organ damaged biopsy when possible. **Results.** The patient series included 14 men and 15 women. The median ages was 67 years (39-85) with 14 patients younger than 65. The monoclonal component (MC) isotype was IgG in 34. 4% of cases, IgA in 20. 7% and micromolecular in 44. 8%; the median proteinuria was 5. 1 g/24h (0. 1-13. 6); the bone marrow plasma cell infiltration was >10% in 13 patients. The Mayo risk score was good-intermediate in 16/29 patients (55%) and high in 13/29 patients (45%). At diagnosis, organ involvement was: 89% renal, 45% cardiac, 20% hepatic, 10% tongue, 3% pulmonary. As first line therapy, 21 patients received MD or MP, 3 of whom achieved a complete hematologic response (CR) and 4 a partial response (PR); 4 of them obtained a partial organ's response. Eight patients had a stable disease (SD) and 6 progressive disease (PD). Among the 7 patients who underwent HDM plus SCT: 2 obtained a CR with a partial organ response, 1 a PR, 3 had a SD and 1 PD. Thereafter, 8 patients were treated with second-line therapy: 5 with Bortezomib-, 1 with Lenalidomide-, 1 with Thalidomide-based regimens and 1 with MD. Three of the latter patients

obtained a partial hematologic and organ response, 2 a SD and 3 showed PD. The median follow-up was 20 months (range 1-123), with an overall survival (OS) and a progression-free survival (PFS) rate at 2 years of 76% (95%IC 60-97%) and 58% (95%IC 41-84%), respectively. With regard to prognostic factors, thrombocytosis, plasma cell bone marrow infiltration, age, cardiac involvement, hypoalbuminemia and the Mayo risk score were analyzed by the log-rank test. Only the Mayo risk score proved statistically significant, with 2-year OS and PFS rates of 86% vs 48%(p=0. 002) and 73% vs 42% (p=0. 04), respectively. By multivariate Cox regression analysis, the Mayo risk score remained the only predictor of worse outcome, statistically related to OS (p=0. 02) and PFS (p=0. 0012). **Conclusions.** We confirm the Mayo risk score as the most predictive factor of OS and PFS in patients with AL amyloidosis. Our results allow to consider Melphalan as a valid therapy in selected patients. New drugs and SCT can further improve the outcome of AL amyloidosis patients.

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'NON-PRODUCER' PLASMA CELL MYELOMA MAY SYNTHESIZE UNASSEMBLED IMMUNOGLOBULIN HEAVY CHAIN: CLINICOPATHOLOGIC STUDY OF 4 CASES OF MYELOMA WITHOUT M-SPIKE OR DETECTABLE CYTOPLASMIC LIGHT CHAINS

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Background. Plasma cell myeloma without M-spike and detectable cytoplasmic light-chain is rare and has been previously defined as the non-producer variant. Clinicopathologic study of this variant of myeloma is limited to sporadic case reports, and its pathogenesis is currently unclear. We present a retrospective analysis of 4 cases of "non-producer"myeloma. **Aims.** To investigate the clinical presentation, pathological features and clinical outcome of "non-producer"myeloma. **Methods.** After institutional approval, 4 cases of plasma cell myeloma without detectable M-spike or cytoplasmic light-chain were collected. Of 4 cases, 2 were encountered among 512 total cases of myeloma registered at Duke University Hospital within the past 5 years. Clinical information, laboratory tests, pathologic evaluation, treatment and follow-up were retrospectively analyzed. **Results.** Of 4 cases, all are male patients. Median age at diagnosis is 59. 5 years (range 56-80). Three cases presented with mild-moderate anemia/thrombocytopenia, and the remaining case presented with multiple skin nodules. While none had M-spike detected in serum or urine, bone lytic lesions were identified in 3 of 4 cases. Other laboratory tests were relatively unremarkable, except for slightly increased/high normal creatinine/BUN in 2 cases, mildly decreased/low normal serum albumin in 1 case and mildly-moderately decreased serum immunoglobulin profile in 3 cases. All cases demonstrated bone marrow involvement by neoplastic plasma cells, ranging 5-90%, with median of 60%, and morphologically, the neoplastic plasma cells displayed lymphoplasmacytoid features. None showed rouleaux formation or circulating plasma cells. In 2 cases, the neoplastic plasma cells demonstrated relatively scant cytoplasm, raising the possibility of small B-cell leukemia/lymphoma with plasmacytic differentiation. Immunohistochemical analysis showed plasma cells were positive for CD56 in 2 cases and negative in the remaining cases. While none had cytoplasmic light chains detected, 2 cases were positive for cytoplasmic heavy-chain of γ isotype. Two cases expressed cyclin D1, corresponding to *CCND1/IGH* fusion detected by cytogenetic analysis, while the remaining cases were negative. Two cases had treatment and follow-up information available. One patient with solitary skin plasmacytoma was treated with radiotherapy but had a poor response and apparent disease progression 5 months after diagnosis. The other patient was treated with combined chemotherapy supported by autologous stem cell transplant and was alive with relatively normal laboratory tests 26 months later. **Conclusions.** "Non-producer"myeloma is extremely rare, comprising ~0. 4% of total myeloma cases per our data. While our cases showed no evidence of M-spike or significantly compromised renal function, as reported in non-secretory myeloma, they demonstrated other features suggestive of myeloma, including lytic bone lesions and/or reduced polyclonal immunoglobulins. Due to negative M-spike and lymphoplasmacytic morphology in some cases, the differential diagnosis may include small B-cell neoplasms with plasmacytic differentiation, which can be excluded by immunophenotypic analysis. Although cytoplasmic light-chain was absent in all 4 cases, expression of cytoplasmic heavy-chain of γ isotype in 2 of our cases raises the possibility that some of the previously defined "non-producer"myelomas may actually synthesize unassembled heavy chains. The clinical outcome of this variant requires further analaysis, including investigation of additional cases and longer follow-up.

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COMPARISON OF ESTABLISHED AND NEW SERUM FREE LIGHT CHAIN ASSAYS FOR THE DIAGNOSTIC ASSESSMENT OF MYELOMA KIDNEY

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Background. The development of sensitive immunoassays (Freelite®) for the quantification of serum free light chains (FLCs) has led to a paradigm shift in the diagnosis, assessment and monitoring of patients with plasma cell dyscrasias; highlighted by their inclusion in international guidelines. Acute kidney injury (AKI) secondary to myeloma kidney affects around 10% of patients with myeloma, and has a profound impact on patient morbidity and mortality. Improving outcomes in these patients relies on the rapid diagnosis and initiation of disease specific treatment. Recently, new immunoassays which use monoclonal antibodies, opposed to the original polyclonal antibodies, against FLCs have become commercially available. The purpose of this study was to compare the diagnostic sensitivity of these new monoclonal assays with the established polyclonal FLC assays in the context of individuals presenting with new severe AKI secondary to multiple myeloma. **Methods.** Sera from 28 patients with AKI secondary to multiple myeloma (17 lambda, and 11 kappa monoclonal proteins) was stored at -80ºC until thawed for the current study. Serum κ and λ FLC concentrations were measured by nephelometry, on a Dade-Behring BN®II Analyser, using particle-enhanced, high-specificity, homogeneous immunoassays (Freelite®, The Binding Site Group Ltd, Birmingham, UK) and monoclonal N-Latex FLC assays (Siemens Healthcare Diagnostic Products GmBh, Germany). **Results.** There was poor agreement between the two assays for the absolute values for both kappa (r^2=0. 87) and lambda (r^2=0. 3), neither reaching the required r^2=0. 95 by the clinical and laboratory standards institute (CLSI) guidelines. 5/28 (18%, Table 1) of patients were misclassified using the N-Latex assay with serum values below the nephrotoxic threshold of 500mg/L (median 221mg/L, range 1-493mg/L). In 1/17 patients the N-latex assay failed to identify monoclonal lambda light chain (Freelite=1810mg/L vs. N-Latex FLC=0. 5mg/L). **Conclusions.** A rapid identification of high levels of monoclonal FLCs in patients with AKI can allow earlier disease specific treatment and improved patient outcomes. The new N-Latex assay does not clearly identify patients at risk of AKI (FLC >500mg/L) and it can miss monoclonal lambda FLCs.

Table 1.

Patient	Multiple myeloma type	Freelite			N-Latex FLC			Missed by N-Latex FLC	Misclassified by N-Latex FLC as <500mg/L
		κ	λ	Ratio	κ	λ	Ratio		
2	Free λ	2.00	7010.0	0.0003	1.02	322.00	0.003	No	Yes
7	IgA λ	8.05	1810.0	0.0044	4.19	0.52	8.058	Yes	Yes
10	IgG λ	8.47	1080.0	0.0078	8.64	64.30	0.134	No	Yes
13	IgG λ	9.25	572.0	0.0162	9.31	225.00	0.041	No	Yes
23	IgG κ	796.0	6.33	125.75	493.00	16.60	29.699	No	Yes

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VELCADITO: A MODIFIED VISTA SCHEME WITH VERY LOW DOSES OF BORTEZOMIB FOR THE TREATMENT OF FRAIL PATIENTS WITH MULTIPLE MYELOMA

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Introduction. Patients diagnosed multiple myeloma (MM) older than 75 years old are infrarrepresented in clinical trials, therefore, chemotherapy in these patients has not been well-stablished. Bortezomid, melphalan and prednisone (VISTA trial) are widely used in these frail patients and it is associated with considerable neurological toxicity (overall: 44%, grade III-IV> 13%) being necessary bortezomib dose reduction and affecting quality of life of these elderly patients. **Aims.** Evaluate the efficacy and tolerability of a new scheme with very low doses of Bortezomib (Velcadito) in elderly patients diagnosed with MM. **Materials and Methods.** Velcadito scheme consisted on monthy cycles of bortezomib: 1. 0 mg/m^2, exclusively on days 1 and 4. Melphalan (9 mg/m^2) and prednisone (60mg/ m^2) were also administered on days 1 to 4 of each cycle. Only on first cycle, bortezomib was administered at dose of 1. 3 mg/ m^2 on 1, 4, 8 and 11 days. **Results.** Between 2008 and 2012, nineteen patients received Velcadito for the treatment of MM. 74% as first line and 26% as second of subsequent lines. Median age at treatment was 77 years old (68-71), and the median cycles received were 9 (2-16). Focussing efficacy, overall response rate

was 78,95% (26,32% complete response; 10,53% very good partial response; 42,11% partial response). Stable disease was achieved on 21,05% patients. Considering neurological toxicity 16% suffer from any grade of neuropathy, with only 1 patient (5%) with grade III-IV, developed in the fist cycle of bortezomib. The progression-free survival and overall survival was 10 (5-54) and 11 (5-57) months, respectively. **Conclusions.** Treatment with Velcadito scheme is feasible in elderly patients with overall response rates comparable toVISTA trial and with low neurological toxicity. This scheme show promising results and should be considered in elderly patients diagnosed with MM.

Table 1.

Median age	77
Line	
1°	74%
2°	26%
Responses	
ORR	78,9%
CR	26,3%
VGPR	10,5%
PR	42,1%
SD	21,0%
PFS	10
OS	11

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CLINICAL FEATURES OF HIGH-RISK MULTIPLE MYELOMA: A SINGLE CENTRE RETROSPECTIVE STUDY

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Background. The outcome of multiple myeloma has improved with the advent of novel drugs; nevertheless, some patients present refractory multiple myeloma or exhibit early relapse after an initial response. Ultra high-risk myeloma, defined as myeloma leading to death within 24 months, affects 15%-20% of patients (pts). **Aims.** The aim of the study was to evaluate the impact of diagnostic phase clinical and laboratory ultra high-risk myeloma parameters (HRM) on Response Rate (RR). **Materials and Methods.** Between October 2005 and March 2011, 14 pts with HRM (2 M (21%), 11 F (79%), median age 66. 5 years, range 51-81) were retrospectively selected from among 120 consecutive pts with de novo multiple myeloma. . According to ISS, 64% (9/14 pts) were in stage III, and 36% (5/14) in stage II; M protein type was IgG in 64% (9/14), IgA in 7% (1/14), BJ in 29% (4/14); a skeletal survey (with conventional skeleton radiography and magnetic resonance imaging) was done in all pts; 7% (1/14) presented extramedullary disease, abnormal LDH was present in 35% (5/14), 50% (7/14) showed bone marrow plasmacellular involvement >50%; 21% (2/14) showed a low (<1500/µl) absolute lymphocyte count; 93% (13/14) showed CD56 positivity at flow cytometric immunophenotypic analysis. Baseline treatment was Melphalan-Bortezomib-Prednisone in 50% (7/14), Bortezomib-Thalidomide-Dexamethasone in 14% (2/14), Bortezomib-Dexamethasone in 21% (3/14), Melphalan-Prednisone in 7% (1/14), Vincristine-Adriamycin-Dexamethasone in 7% (1/14). Response rate was: Partial Remission in 14% (2/14 pts) and Progressive Disease in 86% (12/14 pts) with 98% (13/14) of deaths due to progressive disease. Median overall survival was 11 months (range 1-26). **Results.** At univariate analysis a statistically significant correlation (p=0. 03) was observed between RR and the baseline lymphocyte count; no significant correlation was found with the other clinical and laboratory characteristics analyzed. **Conclusions.** Ultra high-risk myeloma affects a rare subset of patients that are difficult to recognize at diagnosis on the basis of standard staging systems and conventional prognostic factors. Our results indicate that a low absolute lymphocytic count at diagnosis can identify a subgroup with better prognosis among this cohort of patients and could be a useful tool for the characterization of HRM patients. Further studies in larger populations are needed to confirm our results.

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ANALYSIS OF THE RESPONSE RATE BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN PATIENTS WITH MULTIPLE MYELOMA, COMPARING THREE DIFFERENT INDUCTION REGIMENS

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Introduction. The chemotherapy regimen most commonly used until recently for the induction of MM patients, eligible for ASCT, was (VAD) doxorubicin + vincristine + dexamethasone. The overall response (OR) rates, obtained after induction, reached 50% in some studies that used VAD as induction. The use of new drugs, such as thalidomide, has shown a significant improvement in the induction response rate, reaching an OR above 80%. This response may reflect better event-free survival and better overall survival. In addition to all these factors, besides being the only novel agent available in the public health system, the oral mode of use, ease and low cost make thalidomide a desirable drug to combine with other drugs for induction regimens. **Aims.** To compare the response rates among three different induction regimens after pre-ASCT: TD (thalidomide + dexamethasone) vs. CTD (cyclophosphamide + dexamethasone + thalidomide) vs. VAD in patients undergoing ASCT. **Methods.** A retrospective cohort of patients with MM undergoing ASCT at a specialized hematology treatment center among January 2007 to June 2011 was studied. We included patients who underwent VAD-induction regimens (vincristine 0. 4mg/d/4 days+ doxorubicin 9mg/m2/d/4 days+ dexamethasone 40mg/d-1-4, 9-12, 17-20), CTD (cyclophosphamide 50mg/d / continuous + thalidomide 100-200mg/d-continuous+ dexamethasone 40mg/ weekly) and TD (thalidomide 100-200mg/d-continuous + dexamethasone 40mg/ weekly), and excluded patients who had received prior to ASTC more than six cycles or regimens other than those above. Statistical analysis was performed with SPSS 15. 0, through comparative analysis with ANOVA and Kaplan-Meier curves for survival, considering p <0. 05 as significant. **Results.** A total of 80 MM patients submitted to ASCT was elected. Seven patients were excluded, with 73 analyzable patients remaining. The patients were 56% male. The median age at diagnosis was 55 years old (28-68). The immunoglobulin subtypes at diagnosis were: IgG = 50%, IgA = 23%, 19% = light chain, IgM = 1. 4% and non-secreting = 3%. The Durie-Salmon staging was: IIIA+B=88% and IIA+B=12%. The numbers by induction + mobilization scheme were: VAD+(Cy+GCSF)=17 cases, VAD+GCSF=10, CTD+GCSF=14 and TD+GCSF=31. The median number of induction cycles was 3 (1-6) for VAD and 4 (3-6) for TD and 4(2-4) for CTD. The response rates above very good partial response (VGPR) after induction, were higher in the TD group (36%) and CTD (50%) group than in the VAD group (14%), (p=0. 039). (See Table 1.) The progression-free survival at 5 years for the entire group of patients was 43%, without difference between groups. (p=0. 27) The overall survival rate at 5 years was 71%, without difference among the groups, (p=0. 41). **Conclusions.** Patients who received CTD and TD as pre-ASCT induction regimens achieved better response rates above VGPR, when compared with the VAD regimen. (P = 0. 039). The CTD group showed a better response than the TD group, albeit without significance, but we attribute this to small cohort. An increased number of patients will probably show a significant difference between the CTD and TD groups.

Table 1. Response rate between three different induction regiments in MM patients.

	VAD	TD	CTD	TOTAL
CR	1 (4%)	3 (10%)	0	4
VGPR	3 (10%)	8 (26%)	7 (50%)	18
PR	17 (61%)	11 (35%)	3 (21%)	31
MR	0	0	1 (7%)	1
Not avaliated	7 (25%)	9 (29%)	3(22%)	19
TOTAL	28 (100%)	31 (100%)	14 (100%)	73

p=0.039

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MEASUREMENTS OF SERUM INVOLVED FREE LIGHT CHAINS AFTER ADMINISTRATION OF ZOLEDRONIC ACID IN MULTIPLE MYELOMA PATIENTS SHOW DIVERSE CHANGES

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Background. Aminobisphosphonates (aBP) are effective in the prophylactic treatment of the osteolytic bone disease seen in patients with multiple myeloma (MM). Treatment with aBPs leads to a decrease in skeletal related events. However, there are ongoing discussions as to whether or not aBPs have an anti-myeloma effect as well. Several *in vitro* studies have shown anti-myeloma effects, but only recently this has been confirmed in a clinical study, in which patients receiving zoledronic acid (ZOL), intravenously, had significantly increased overall survival compared to patients receiving orally administered clodronate. Other studies comparing pamidronate to placebo has failed to confirm a prolonged survival. Serum free light chain (sFLC) is a biomarker in MM, with a short serum half-life of 2-6 hours. Theoretically, the measurement of sFLC can be used as an early indicator of anti-myeloma activity. **Aims.** To assess whether measurements of sFLC, can demonstrate an anti-myeloma effect of the aBP, ZOL. **Methods.** Fifteen patients with clinically stable MM (based on stable levels of serum M-protein and immunoglobulins) absent from anti-myeloma therapy, were included. Informed consent was obtained. Mean age 67. 7 years (43 - 92 years). All patients received prophylactic treatment with ZOL, 4 mg i. v. Seven (47 %) patients were treated with ZOL for the first time. We collected serum samples before treatment with ZOL, after 4-6 hours, 1, 2, 3 and 4 days. A significant change in sFLC at a 95 % level was calculated as 1. 96 * $\sqrt{2}$ * $\sqrt{CVtotal^2}$. **Results.** Four (27 %) patients had a significant decrease in involved FLC (iFLC). In two patients the decline was transient. In 6 (40 %) patients no change were seen neither in iFLC nor the uninvolved FLC. In 2 (13 %) patients there was a significant rise in iFLC on day 5. Two (13 %) patients had a short rise in iFLC, falling back to baseline values. There were no differences in the results obtained from patients who had received ZOL before and the aBP naïve patients. **Summary and Conclusions.** Using the sensitive biomarker sFLC during treatment with ZOL we observed a significant decline in iFLC in a minor fraction (27%) of patients within few days after the administration. In 2 patients we observed a significant increase iniFLC. No change was observed in most of the patients. Thus, even though we observed a decline in 4 patients, there was no “clear picture”in the iFLC changes. Oppositely, the response in 4 patients could indicate an anti-myeloma effect of ZOL in some patients. Moreover, the potential anti-myeloma effect of ZOL may be dependent on a synergistic effect of another, simultaneously given, anti-myeloma therapy.

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NUMBER NEEDED TO TREAT (NNT) AS A MEASURE OF DRUG BENEFIT: LENALIDOMIDE VS. BORTEZOMIB AS INITIAL TREATMENT FOR MULTIPLE MYELOMA (MM)

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Background. Lenalidomide (LEN) and bortezomib (BORT) are active agents for the initial treatment of MM. LEN is administered 25 mg/day orally on days 1-21 of repeated 28-day cycles. The dosage of BOR is 1. 3 mg/m^2 intravenous dose on days 1, 4, 8 and 11 for eight three week cycles. Both agents have demonstrated an improvement in disease response, progression free (PFS) and overall survival (OS) as initial therapy in MM. NNT represents the number of patients that need to be treated with a new intervention in order to have one additional patient deriving benefit, and is a recognized and widely used to further interpret clinical benefits from randomized trials. **Aims.** In this analysis, the NNT approach was used to compare LEN and BORT as initial therapy in MM. **Methods.** The randomized trials for LEN (Zonder, 2010) and BORT (San Miguel, 2008) were reviewed. A key requirement for a NNT comparative analysis is that all outcomes be measured against a comparable control in similar patients. The NNT was then calculated for both agents with respect to disease response and PFS at 12 months by taking the reciprocal of the absolute differences in treatment effect between the experimental therapies and the control group. **Results.** For a response, LEN and BORT had a comparable NNT of 3 to 4 patients; in order to have one patient achieving a disease response. However, a difference in the NNT was noted for PFS at 12 months; only 4 patients would need to be treated with LEN for a patient remaining progression free compared to 6 with BORT. **Conclusions.** In situations of multiple numerical outcomes from randomized trials, the NNT approach is a simple and effective method to express the findings in a clinically meaningful way. In this analysis, it appears that patients treated with LEN are more likely to respond and to achieve a 12 month PFS than comparable patients treated with BORT. Possible reasons for this effect include differences in the trial population, extent of disease, and/or true differences in efficacy.

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LENALIDOMIDE OR BORTEZOMIB FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM): A COMPARATIVE EFFECTIVENESS ANALYSIS USING INDIRECT STATISTICAL TECHNIQUES

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Background. Lenalidomide (LEN) and bortezomib (BORT) are both effective for the treatment of patients with relapsed/refractory MM. The former is administered 25 mg/day orally on days 1-21 of repeated 28-day cycles. The latter as a 1. 3 mg/m^2 intravenous dose on days 1, 4, 8 and 11 for eight, three week cycles. Currently, there are no data available from a head to head randomized phase III trial comparing LEN and BORT. **Aims.** In the absence of such data, an indirect comparison between LEN and BORT was performed in the relapsed/refractory MM setting. Such an analysis was feasible because comparable controls were used in the pivotal randomized trials and patients had similar baseline characteristics. **Methods.** Three pivotal randomized trials with LEN (n=2) and BORT (n=1) in the relapsed/refractory setting were identified. Patients within each trial had similar disease characteristics. Data in terms of response rate (RR), time to progression (TTP) and overall survival (OS) were extracted from the pivotal trials. An indirect statistical comparison between LEN and BORT was then performed on these endpoints using the method of Bucher et al. (1997), which partly maintains the benefits of randomization on the magnitude of benefit. **Results.** The analysis identified significant differences in efficacy between these drugs. Patients treated with LEN were significantly more likely to achieve a disease response (OR=1. 92; 95%CI: 1. 15 - 3. 20) and to have a prolongation in TTP (HR = 0. 64; 95%CI: 0. 44 - 0. 91). The analysis also identified a trend for an OS benefit in patients receiving treatment with LEN over BORT (HR = 0. 71; 95%CI: 0. 46 - 1. 11). **Conclusions.** Keeping in mind the caveats associated with cross trial comparisons, this indirect evaluation suggested increased effectiveness of LEN over BORT in MM patients with refractory/relapsed disease. These findings, along with its oral route of administration and established safety profile suggest that LEN should be the preferred agent for refractory/relapsed MM.

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CASE SERIES OF MULTIPLE MYELOMA PATIENTS WITH SOLITARY PLASMACYTOMAS

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Background. Some patients with multiple myeloma (MM) has additional tumor masses that can affect various organs and worsen prognosis and treatment outcomes. Myeloma growth on extramedullary sites is a sign of more aggressive biological behavior of tumor. Some prognostic factors that could predict spread over bone marrow (BM) may be useful to initial stratification of patients to more aggressive therapy. **Aims.** To investigate possible correlations between laboratory findings and clinical outcome in patients with newly diagnosed symptomatic myeloma. **Methods.** Eighteen adult patients (median age - 55. 9 years; male - 11) with newly diagnosed MM were assessed. The diagnosis of active MM was made according to standard diagnostic workup (history and physical exam, serum albumin, quantitative immunoglobulins, beta-2 microglobulin, serum and urine electrophoresis, serum and urine immunofixation, serum and urine free light chain assay, BM aspiration and biopsy, BM multicolor flow cytometry (MFC) analysis with documentation of an aberrant surface phenotype of plasma cell (PC) population, plasmacytoma immunohistochemistry (IHC), BM cytogenetics and FISH, skeletal sur-

vey, computed tomography (CT), magnetic resonance imagine (MRI), or positron emission tomography (PET)/CT scan. We also studied their clinical findings with use of skeletal survey, CT, MRI and PET/CT during treatment phase and follow-up period. The BM MFC was performed at time of initial diagnosis. MM disease stage was assessed using Durie-Salmon and International staging systems. All the patients were treated with initial bortezomib-based therapy and bisphosphonates. Responses were investigator-assessed and based on EBMT and IMWG response criteria. **Results.** Seven patients (38. 9%) had solitary plasmacytomas at diagnosis which were detected using CT, MRI, PET/CT (3, 3, and 1 cases, respectively). Six patients (85. 7%) had osseous plasmacytomas, and 3/7 (42. 9%) - multiple (≥2) osseous plasmacytomas, whereas one patient had extraosseous (both breasts) plasmacytomas. Six/7 patients (85. 7%) was operated on; 5/7 (71. 4%) manifested with signs of vertebral column instability or spinal cord compression and was operated on before start of primary therapy. One patient (14. 3%) was operated on during primary therapy. During follow up period (2 years after first admission) 1/7 patient was diagnosed as having new osseous plasmacytoma which was successfully operated on. MFC was performed on 18 consecutive BM aspirates: CD38 and CD138 was expressed in 18 cases (100%), CD19 - 4 (22. 2%), CD56 overexpression - 5 (26. 3%), CD117 - 7 (38. 9%), CD20 were negative in all cases. Patient' PC phenotype detected on BM MFC and results of plasmacytoma IHC were identical. All the patients with plasmacytomas did not show CD117 expression (results of BM MFC and IHC assays). **Conclusions.** Our study shows that the presence of plasmacytomas correlate with the absence of CD117-expression of atypical PCs in patients with newly diagnosed symptomatic myeloma. There were not revealed any other correlations between results of laboratory studies and clinical course in our patients. These data demonstrates that the newly diagnosed patients with active myeloma require a very thorough evaluation including CT, MRI and/or PET/CT (preferable diagnostic tool) to rule out the presence of solitary plasmacytomas. Further studies are needed to confirm this proposal.

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ABSOLUTE LYMPHOCYTE COUNT IS NOT A PREDICTOR FOR SURVIVAL IN NEWLY DIAGNOSED ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Background. Absolute lymphocyte count (ALC) > 1. 400 x10^9/l, as a surrogate marker of host immune status, has been reported to be an independent prognostic factor for clinical outcome in patients with previously untreated multiple myeloma (MM). However, most of the patients evaluated received stem cell transplantation (SCT); less evidence is available on elderly patients or those unable to SCT. **Aims.** To evaluate retrospectively the correlation between ALC, detected at the time of MM diagnosis, and clinical outcomes (first remission rate and overall survival) in patients older than 65 y. o. or those not eligible to SCT. **Methods.** Between 1998 and 2006, 103 consecutive patients were evaluated among four institutions; none of patients was neither uniformly treated nor part of a clinical trail. The primary endpoint was to assess the role of ALC, at the time of MM diagnosis, on overall survival (OS); secondary outcome was the correlation between ALC and rate of first complete remission. The OS was measured, by Kaplan and Meier analysis, from MM diagnosis to death or last follow-up. Patients that were lost to follow-up were censored in the survival analysis. Differences between survival curves were tested for statistical significance using the two-tailed log-rank test. **Results.** The median age for this cohort of 103 MM patients was 69. 4 years (+ 10. 8 years). Baseline characteristics (ISS, LDH, M Component, Haemoglobin Levels and Calcium) where not statistical significant. Three patients were lost to follow-up. Most of patients were treated with regimens containing Melphalan, Prednisone with/without Thalidomide. The median follow-up was 24. 3 months (range: 1-81 months). At the time of the analysis 17 patients had died. Of the 103 patients, 7 (7%) of the patients died of MM. ALC, as a continuous variable, was not identified as prognostic factor for overall survival (OS) (ALC < 1. 400 x10^9/l vs ALC > 1. 400 x10^9/l = HR 1. 214 (95%CI: 0. 222-6. 650; p=0. 823]). OS of MM patients with ALC > 1. 400 X10^9/l was 82 months vs 76 of those with ALC < 1. 400 x10^9/l (p= 0. 23) (Figure 1). ALC did not influence response rate of first complete remission (ALC < 1. 400 x10^9/l vs ALC > 1. 400 x10^9/l = HR 1. 538 [95%CI: 0. 662-3. 570; p= 0. 317]). **Conclusions.** This study showed that, in newly diagnosed MM, ALC is not an independent prognostic factor for OS and does not influence rate of first complete remission.

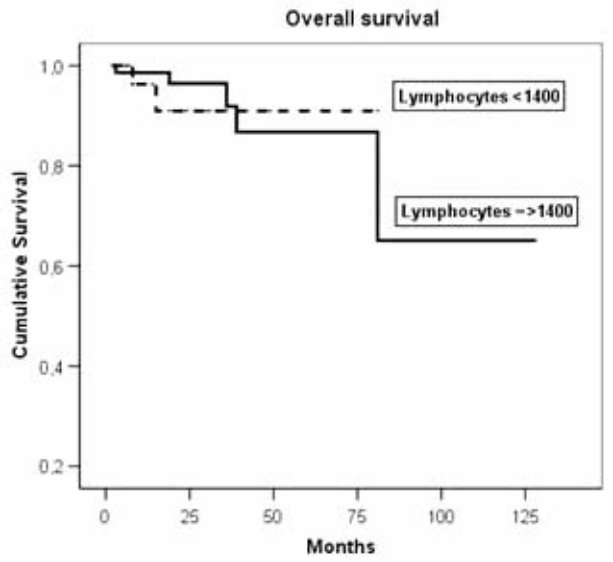

Figure 1.

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IS INTERFERON-ALPHA REALLY DEAD AS THERAPY FOR MULTIPLE MYELOMA PATIENTS?

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Background. The use of Interferon (IFN)-alpha for the induction and maintenance treatment in patients with multiple myeloma (MM) has yielded discordant results. The most important role of IFN is in responding patients when used as maintenance therapy after induction treatment. An overall modest prolongation in response duration, reported in the majority of studies, and the development of new agents have put this drug into disuse. However, in our clinical practice we have observed MM patients with a long overall survival (OS) when treated with IFN as maintenance therapy. **Aims.** We hereby analyzed the efficacy and toxicity of IFN used as maintenance therapy in MM patients over a prolonged time period. **Methods.** A retrospective analysis was conducted on 38 MM patients who received IFN as maintenance therapy for more than 24 months. **Results.** The patient series included 24 men and 14 women. The median age of the patients was 58 (range 35-74) years. Twenty-five were IgG, 8 IgA and 5 had light chain MM. Twenty-four and 14 had Durie and Salmon stages III and I/II, respectively. Two patients had serum creatinine levels >2 mg/dl. Twenty-nine patients had received at least one line of therapy and 9 >two lines. Twenty-eight have undergone an autologous stem cell transplant: one in 21 patients and two in 7. After induction therapy, the disease status was as follows: 15 patients were in complete remission (CR), 5 in very good partial response (VGPR) and 18 in partial response (PR). The median follow-up before starting IFN therapy was 13 months (range 3-54). IFN was administered as a single agent in 32 patients and in association with steroids in 6 patients. The weekly dose of IFN administered to all patients was 9 MU (3MU three times a week). The median duration of IFN maintenance was 68 months (range 13-242). After a median follow-up of 153. 5 (range 33-307) months, 8 patients continue IFN treatment, while 30 patients have discontinued: 12 for disease progression, 6 for toxicity, 1 for non-compliance, 1 for a second neoplasia after 45 months, 1 for a cerebral stroke and 9 for other causes. The OS rate at 12 years is 78%. The most frequent side effects recorded in our patients were flu-like syndrome **(8%) and hepatotoxicity (8%). Conclusions.** Today, IFN is rarely utilized in the management of MM patients. Nonetheless, our report indicates that some patients can witness a prolonged OS following the use of IFN as maintenance therapy. In our experience, the drug proved well tolerated and its toxicity, reported in the majority of studies, maybe overcome with the use of the pegylated form, at the moment under evaluation. It will be important to try to identify pre-

dictive factors that can help to recognize MM patients who can benefit from IFN maintenance treatment.

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BORTEZOMIB AND HIGH-DOSE MELPHALAN AS CONDITIONING REGIMEN BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA: EXPERIENCE IN A UNIVERSITARY HOSPITAL

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Background. The combination of bortezomib and high-dose melphalan (HDM) is an attractive approach to improve the efficacy of the conditioning regimen. Furthermore, this association was expected to be safe because bortezomib and melphalan do not share common toxicities (mainly neurologic toxicity for bortezomib and hematologic toxicity for HDM). We followed a phase 2 study of the Intergroupe Francophone du Myélome (IFM) (Blood 7 Jan 2010, 115 (1); 32-36). **Aims.** 1) to evaluate response rates after intensive therapy and to assess the toxicity of this new conditioning regimen. 2) To know the toxicity. **Methods.** 11 patients (5 males/6 females, median age 59 years (34-73). Myeloma type: 3 IgG, 5 IgA, 2 Bence Jones, and 1 plasmatic cells leukemia; Durie-Salmon: 3 IIA, 5IIIA, 2 IIB) with multiple myeloma were treated between February 2010 and February 2012 to receive bortezomib (1 mg/m2 × 4) and melphalan (200 mg/m2) as conditioning regimen (Bor-HDM). All patients received induction treatment with VCD (Bortezomid 1,3 mg/m2 D1, D4, D8, D11; Cyclophosphamide 600 mg/m2 D1, D8; Dexamethasone 40 mg D1, D4, D8, D11) 6-8 cycles/21 days. Overall, 81,8% of patients achieved at least PR, including 2 patients with CR (18,2%) before ASCT. Bortezomib was administered intravenously at 1 mg/m2 on days -6, -3, 1, and 4. Melphalan was administered intravenously at 200 mg/m2 on day -2. Peripheral blood stem cells (≥ 2 × 106 CD34+ cells/kg) were infused on day 0. Peripheral blood stem cells (median 2,83 × 106 (1. 97-3. 89). CD34+ cells/kg) were infused on day 0. **Results.** All patients received granulocyte growth factors. There was no engraftment failure. Neutrophils (ANC ≥ 0. 5 × 109/L) and platelets (≥ 20 × 109/L without transfusion) recovered in median times of 11 days (range, 11-13 days) and 13 days (range, 11-18 days), respectively. Patients were discharged from the transplantation unit in median times of 27,5 days (range, 21-25 days). There was one treatment-related death, at 11 days of candidiasis (C. glabrata). Some serious adverse events were reported: 1 Salmonella diarrhea, and 1 pneumonia. 5 bacteremias (45,4%) were documented: 4 *Staphylococcus epidermidis*, 1 *Enterococcus faecalis*, 1 *Pseudomonas aeruginosa.* . The most frequently reported grade 3 or 4 nonhematologic toxicities were mucositis of upper and lower digestive tract sites (72,7%). Other grade 1 or 2 adverse events were digestive (diarrhea, 18,2%). It should be noted that peripheral neuropathy (PN) was present at the time of ASCT,1 patients and did not get worse after Bor-HDM treatment. At time of reporting, median follow-up time from induction therapy was 24 months (range, 8-30 months). 2 patients relapsed at 3 and 9 months after ASCT, and they died of progressive disease. Evaluation of response rate at 100 days after ASCT have not changed. **Conclusions.** Our experience shows that bortezomib can safely be combined with HDM as a preparative regimen followed by ASCT. This regimen was well tolerated with no increased toxicity. Engraftment was not affected by the addition of bortezomib. PN did not worse after this conditioning regimen.

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STUDY OF EFFICACY RECOMBINANT HUMAN ERYTHROPOIETIN IN MULTIPLE MYELOMA PATIENTS WITH ANEMIA

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Background. Anemia in Multiple Myeloma (MM) patients can influence the efficacy of chemotherapy, survival rate and overall quality of life (QoL). Red blood cell (RBC) transfusions are routinely used to treat anemia. While recombinant human erythropoietin (rHuEPO) treatment has been shown to significantly increase hemoglobin (Hb) concentration, reduce the number of RBC transfusions and improve QoL in patients with chemotherapy induced anemia. **Aims.** To study efficacy rHuEPO (Epoetin alpha) in MM patients with anemia. To compare the results of treatment MM patients with anemia who had received only chemotherapy versus patients who had received chemotherapy and rHuEPO. **Methods.** There was done prospective study to investigate the effectiveness of rHuEPO therapy (n=65) and was compared to control group of patients (n=31) who hadn't been received rHuEPO. All patients (n=96) had been diagnosed multiple myeloma in second or third stage (by WHO classification). Anemia was determined in every patient (Hb level 5. 3-10. 0 g/dL). The median age of patients was 66. 5 years (range 26-84). If Hb concentration was <8. 0 g/dL, the patients were performed RBC transfusions before rHuEPO treatment. Epoetin alpha was injected subcutaneously on 40. 000 IU weekly (n=44) or on 10. 000 IU 3 times a week (n=21). Before start of rHuEPO treatment all patients have being received two or more cycles of chemotherapy (PAD, VD, MP or CVMP). The target Hb level was 12 g/dL and planned duration of rHuEPO therapy was within 16 weeks. The control group of patients was observed during 20 months. The positive response in both group was estimated as increasing Hb concentrating on 2. 0 g/dL or more and so achieving target Hb level (12 g/dL) during the period of rHuEPO therapy. **Results.** In the rHuEPO group of patients (n=65) mean baseline Hb concentration was 8. 87±1. 27 g/dL (median 9. 20 g/dL) and RBC count was 2. 80±0. 46×10^{12}/L (median 2. 85×10^{12}/L). 15 patients had Hb concentration <8. 0 g/dL (5. 3-7. 9 g/dL) therefore they were transfused 2-6 units of red blood until Hb increased up to 8. 0-9. 0 g/dL. The period of rHuEPO-therapy was from 4 to 16 weeks (mean 10. 0±3. 8 weeks). During the study period in the rHuEPO group the positive response was at 42 patients (64. 6%). Only 5 patients (33. 3%) were kept on RBC transfusion dependence. In whole rHuEPO group Hb concentration and RBC count increased from baseline to 11. 41±1. 96 g/dL (p<0. 01) and 3. 58±0. 75×10^{12}/L (p<0. 01), respectively. The patients with positive response more significantly increased Hb level from 9. 00±1. 14 to 12. 35±1. 31 g/dL (p<0. 001) and RBC count increased from 2. 82±0. 38×10^{12}/L to 3. 91±0. 51×10^{12}/L (p<0. 001). However, in control group patients positive response observed at 22. 6% (7 from 31). There were not significant difference of Hb concentration and RBC count in this group of patients (Hb increased from 8. 86±1. 18 to 9. 48±1. 42 g/dL (p>0. 05) and RBC increased from 2. 80±0. 51×10^{12}/L to 3. 01±0. 63×10^{12}/L (p>0. 05)). Besides, during the study period 6 (60%) from 10 patients had been receiving RBC transfusion. **Conclusions.** The study has shown that rHuEPO is effective at increasing Hb concentration, count of RBC and reducing RBC transfusion in MM patients with anemia.

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ERYTHROCYTE SEDIMENTATION RATE CANNOT BE USED TO RELIABLY EXCLUDE MULTIPLE MYELOMA

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Background. Early diagnosis is important in multiple myeloma to enable timely treatment and prevent end organ damage such as renal failure, bone lesions, hypercalcaemia and bone marrow failure. We were concerned that recent advice aimed at primary care physicians stated that a normal erythrocyte sedimentation rate (ESR) may be used to exclude a possible diagnosis of multiple myeloma. It was stated that 'normal levels of inflammatory markers are valuable in ruling out a few specific conditions, notably polymyalgia rheumatica, giant cell arteritis, myeloma and infection of hip revisions'. As per the British Committee of Standards in Haematology (BCSH) guideline we believe that all patients with suspected multiple myeloma should be investigated with screening tests including full blood count (FBC), ESR, urea, creatinine, calcium and importantly, electrophoresis of serum and concentrated urine. Furthermore a serum free light chain assay should be performed in any patient in whom there is a strong suspicion of myeloma and serum protein electrophoresis is negative. This assay is particularly important in the diagnosis of light chain only myeloma, non-secretory myeloma and amyloidosis in whom no monoclonal protein would be detected by routine serum electrophoresis. A recent review recommended serum free light chain assay to be used in conjunction with serum protein electrophoresis when screening for the presence or absence of a monoclonal plasma cell disorder such as myeloma. There is little published work investigating the value of inflammatory markers in the diagnosis or exclusion of myeloma. We therefore performed a concise study of our own patients. **Aims.** The aim of this study was to determine the value ofESRin the exclusion of multiple myeloma. **Methods.** The most recent 100 patients diagnosed with multiple myeloma at North Glasgow Hospitals were identified from our registry. Patient demographics, subtype of myeloma andESRvalue at presentation were obtained from the laboratory database. **Results.** ESRvalues were available in 89 cases. Subtypes of myeloma were recorded; IgG 49%, IgA 20%, Light chain myeloma 25%, IgE 1%, Amyloidosis 3%, Non-secretory myeloma1%; ensuring the sample was representative. The local normal range forESRis ≤12mm/hr. We found 10% of all patients to haveESR≤12mm/hr (within the strict normal range), 25% of patientsESR≤30mm/hr, 48% of patientsESR≤60mm/hr and 70% of patientsESR≤100mm/hr at time of diagnosis. In particular, all patients with amyloid and non-secretory myeloma had anESR≤30mm/hr and 68% of patients with light chain myeloma anESR≤60mm/hr. **Summary and Conclusions.** The low contribution of light chain to the value ofESRhelps explain these findings. ESRis mainly controlled by fibrinogen and other acute phase markers. The molecular weight of fibrinogen is more than ten times that of light chain, markedly limiting the potential effect a rise in light chain can have onESRvalue. This study demonstrates that a nor-

malESRcannot be used to confidently exclude a diagnosis of multiple myeloma, particularly when there is no monoclonal protein present.

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ANALYSIS OF FREE LIGHT CHAINS (FLCS) LEVEL IN PATIENTS (PTS) SUFFERING FROM MULTIPLE MYELOMA (MM) COMPLICATED WITH AMYLOIDOSIS ANTIBODY LIGHT (AL AMYLOIDOSIS)

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Background. In AL amyloidosis, the amyloid deposits consist of monoclonal immunoglobulin light chains, which are producted by plasma cell clones. **Aims.** The aim of the study was to investigate the relationship between the serum kappa and lambda FLCs and the development of AL amyloidosis in pts suffering from MM. **Materials.** The investigations included 70 pts with MM, 40/30 F/M aged 28-83. In 37 pts MM was diagnosed recently, 33 persons were treated from 2 to 34 months. IgG myeloma was diagnosed in 34 pts, IgA in 16, IgM in 4, light chains disease in 6, non-secretory in 6, solitary plasmocytoma in 4 pts. Fifteen pts developed kidney failure. **Methods.** Amyloidosis was diagnosed on the basis of green-tinged amyloid deposits under a polarized light microscope in the adipose tissue from the abdominal fold. The level of serum FLCs was measured by means of the immunonephelometric method and expressed in mg/l. **Results.** Amyloidosis AL was diagnosed in 18 (25. 7%) pts suffering from MM, 9/9 F/M. In 6 (33%) persons with amyloidosis MM was freshly diagnosed, 12 (67%) revealed progression or recurrence of the disease. sFLCs κ level in 18 pts suffering from MM complicated with amyloidosis ranged from 0. 3 to 4780 (x=854. 5. SD=1289) and was higher (p=0. 039) than in the group without this complication: 0. 3 to 426. (x=68. 9. SD=98. 1). The highest sFLCs kappa level was in the group of 5 pts with MM complicated with amyloidosis and kidney failure; it was higher (p= 0. 0008) than in the group with kidney dysfunction but without amyloidosis and higher (p= 0. 001) in comparison to patients with amyloidosis and normal kidney function. The lowest level of sFLCs kappa was demonstrated in patients without amyloidosis and without kidney failure. Amyloidosis affected 12/45 pts suffering from myeloma with predominating light chain kappa, more often in IgG kappa myeloma (9/12 pts) than in IgA kappa (1/3 pts) (p = 0. 05). sFLCs λ level in pts suffering from myeloma complicated with amyloidosis and kidney failure, similarly to pts without kidney failure, was higher (p= 0. 05 and p= 0. 04) than in the group of myeloma without amyloidosis, regardless of the kidney function. Amyloidosis affected 6/19 pts suffering from MM with light chain λ. There was no predominance of any kind of myeloma with λ chain in the development of amyloidosis. In the group of 18 pts with amyloidosis, 16 revealed abnormal sFLCs κ levels (88. 8%), 14 (77. 8%) abnormal sFLCs λ. In the group of 52 pts without amyloidosis, abnormal sFLCs κ and λ levels were observed in 44 (84. 6%) and 24 (46. 1%) pts respectively (p= 0. 03). The κ/λ ratio in pts with amyloidosis ranged from 0. 0 to 5235 (x=465. 5. SD=1222) and it was higher (p= 0. 001) than in MM pts without amyloidosis: 0. 0 - 209. (x=26. SD=46). In both subgroups the values were higher than in the control group (p= 0. 0001). **Conclusions.** Patients suffering from MM complicated with amyloidosis reveal higher levels of sFLCs. The highest values of the above parameters were observed in MM pts with amyloidosis and kidney failure.

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A STUDY OF THE CARDIORESPIRATORY LATE EFFECTS IN PATIENTS WITH ADVANCED INTENSIVELY TREATED MULTIPLE MYELOMA

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Introduction. Modern treatment strategies in myeloma have increased life expectancy, but late effects of treatment and disease have not been studied systematically. **Aims.** To define the prevalence, extent and character of cardiovascular and respiratory compromise and its effect on QOL in intensively treated advanced but stable myeloma patients. **Methods.** We present a cross-sectional, exploratory study of patients with myeloma who had received a transplant and at least one subsequent treatment for progressive disease, who are currently in plateau phase. Following informed consent, data collection comprised self-completed questionnaires, full history, clinical examination and relevant investigations. Statistical analysis: Spearman's rank correlation coefficient was used. Ethical approval: obtained from the Sheffield Ethics Research Committee. **Results.** 32 patients were recruited: 17 males; 15 females. Median age was 61 years (range 41-73); median duration from diagnosis 5. 5 years (range 2-12). Many patients had cardiovascular risk factors such as smoking and hypercholesterolemia, and mean total anthracycline dose was 189mg/m2 (range 0-360mg/m2). There was a significant positive correlation between dependent oedema and years since diagnosis and transplant; pain and pain interference [1] and a negative correlation with physical and role function [2]. 59% of patients also had a raised B-type natriuretic peptide (BNP) (>100pg/ml). 3 patients had abnormal echocardiographs, all of whom had raised BNPs. 3 other patients had abnormal ECGs, though these all had BNPs<100pg/ml. Median dyspnoea score was 33 (IQR 0-67). Respiratory function testing showed 66% and 52% of patients to have FEV1 and FVC (respectively) lower than 90% of their predicted value. 67% and 87% of patients respectively had MIP and MEP values below the predicted range. **Summary and Conclusions.** In this relatively small sample of intensively treated advanced stage myeloma patients there is significant evidence of early cardiovascular dysfunction as evidenced by the high proportion of patients with elevated BNP, with some showing evidence of left ventricular strain on ECG or echo, though this dysfunction as yet remains subclinical. Mild impairment of respiratory function was also frequently found, in spirometry testing (mixed obstructive and restrictive features) and symptomatic questioning. Screening and interventional strategies should be investigated further in the effective management of the consequences of advanced myeloma and its treatment with the aim of prolonging survival and optimising quality of life-
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TREATMENT OF ELDERLY AND FRAIL MULTIPLE MYELOMA PATIENTS WITH LOW DOSE OF LENALIDOMIDE AND DEXAMETHASONE

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The treatment of elderly and frail patients with multiple myeloma is a clinical challenge. Lenalidomide is a very active drug but at the standard dose the toxicity in frail patients is substantial leading to both dose reduction and premature discontinuation of the treatment. Besides the dose also the duration of the treatment with lenalidomide is of great importance. Actually the rate and quality of clinical responses may continue to increase even after the sixth month of therapy. Therefore, a low dose-regimen could improve the safety profile allowing a steady administration of the drug and optimizing treatment outcome of these vulnerable patients. However, data on the clinical effectiveness of low dose lenalidomide regimens are scarce. We retrospectively analyzed a series of twenty relapsed-refractory frail MM patients considered ineligible for a standard dose lenalidomide and who received a lenalidomide dose ≤ 15 mg (l) in association with dexametasone 20 mg (d)Objective of this analysis was to evaluate the Overall Response Rate (ORR), Progression Free Survival (PFS) and toxicity. Patients characteristics are summarized in Table 1.

Table 1.

Patients	**20**
Age, median (range)	**70 (53-82)**
Age>75 (%)	**5 (25)**
Male/female	**11/9**
Comorbidities (%)	
Hepatic	**3 (15)**
Renal	**2 (10)**
Cardiac	**3 (15)**
None	**6 (30)**
STAGE	
I/II/III	**1/0/19**
A/B	**18/2**
Diagnosis IgG-κ/IgG-λ/IgA-κ/IgA-λ	**7/2/4/2**
Micromolecular l/κ	**4/1**
Median previous therapies(range)	**1(1-5)**
Previous ABMT(%)	**6 (30%)**
Dose of lenalidomide	
10mg n (%)	**17 (75)**
15mg n (%)	**3 (15)**
Cycles ld administered median (range)	**8 (1-24)**

Treatment consisted of low dose lenalidomide (10 mg 17 patients; 15 mg 3 patients) days 1-21, and dexametasone 20mg once a week. (ld)All patients were pretreated, 4 relapsed and 16 refractory to the last treatment (median previous therapies =1; range 1-5). Major causes of exclusion from a full dose RD were: age (5 pts; 25%) hepatic dysfuction (15%), renal dysfuction (10%), cardiac dysfuction (15%). The median time from diagnosis of MM to lenalidomide treatment was 37,7 months (range 7-144) and the median time from the last previuos treatment to ld was 13. 6 months (range:3-40 months)Thirteen patients attained a major response (ORR=55%; CR=10%; VGPR=20%; PR=25%), 2 progressed and 5 had a stable disease (35%). With a median follow-up of 13 months, the PFS and OS are 13. 3 months and 14. 7 months respectively. Five pts died due to progression or other causes related to MM. Treatment was well tolerated, with only two discontinuations due to toxicity (1 deep vein thrombosis; 1 diffuse and desquamating rash). Two patients required support with granulocyte colony-stimulating factor (GCSF) to continue treatment. The results of this retrospective analysis show that ld-RD regimen is well tolerated and clinically effective in frail and elderly MM relapsed-refractory patients. In our opinion, in this setting of highly vulnerable patients for whom the relief of symptoms and the containment of the neoplastic clone are major clinical goals, a tailored treatment with ld-RD seems a feasible and advisable therapeutic strategy. . ld= low dose lenalidomide and low dose dexametasone.

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WALDENSTROM'S MACROGLOBULINEMIA:VALIDATION OF INTERNATIONAL SCORING SYSTEM (IPSS)

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Background. Waldenström macroglobulinemia (WM) is defined as lymphoplasmocytic lymphoma with bone marrow involvement and an IgM monoclonal gammopathy of any concentration. International prognostic scoring system for patients (pts) with WM (IPSS-WM) is very important prognostic factor for their survival providing risk-adapted therapy with better outcome. **Aims.** To analyze the clinical and laboratory data and treatment results in 20 pts with WM according to IPSS-WM. **Methods**: Diagnosis of WM was performed according to WHO 2008 classification. IPSS-WM is defined concerning 5 unfavorable factors(age >65 yrs, Hb ≤115 g/l, platelets ≤100x 10^9/l,serum beta 2MG >3mg/l, IgM paraprotein >70g/l) as low risk (≤1 factor except age),intermediate risk (2 factors or only age >65 yrs), or high risk (> 2 factors). **Results.** There were 15 males and 5 females (p<0,05),with Me age 69,5yrs (range 52-80),and 70% of pts over 65 yrs. According to IPSS-WM there were 4 (20%) pts with low,6 (30%) pts with intermediate and 10 (50%) pts with high risk . Symptomatic disease was presented in 35% pts, and 5/7 (71%) of those pts were with high risk IPSS. Also, all pts with hemorrhagic and hyperviscosity syndrome, lymphadenopathy and 90% of pts with organomegaly were in high risk group. Comparing to other risk groups high risk pts were also pts with higher sedimentation rate (60% pts with SE >100) and higher levels of CRP (87,5% pts), serum proteins (50% pts with proteins >80g/l),IgM paraproteins (Me 39,4 g/l),as well as more frequently hypoalbuminemia (50% pts with low albuminemia) and hypogammaglobulinemia (60% pts). Analysis of structure of risk factors in different risk groups showed that the most frequent unfavorable factors were: ≥65,anaemia ≤ 115 g/l and beta 2MG>3mg/l. In this 10yrs retrospective study pts were treated with different therapy modalities,mostly with chlorambucil (40% pts),regimens MP+VMCP (15% pts),FC+FMD (15% pts),COP+CHOP (10% pts) and without therapy in 20% pts. The best therapy response was in low risk group (75% pts with complete and partial response vs. 66,6% in intermediate vs. 30% in high risk group). Median survival was 41 months with overall survival (OS) after 2 yrs in 76,9% and 5 yrs in 29,9% pts. There were statistical significance between different risk groups (low,intermediate,high risk) in 2yrs OS (100%,100%,46,9%) and 5yrs OS (66,7%, 50%, 0%) (p<0,01). **Conclusions.** Our result confirms the literature data concerning the prognostic value of IPSS-WM for survival and risk-adapted therapy in WM pts. It may be useful for defining WM pts for more aggressive treatment including new therapy approaches .

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CLINICAL PRACTICE IN PRIMARY AL AMYLOIDOSIS: QUESTIONNAIRE SURVEY IN HOKKAIDO

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Background. Primary AL amyloidosis is a rare clonal plasma cell disorder characterized by a deposition of monoclonal light chains in various tissues and organs. Except for some relatively fit patients participating clinical trials, current status in clinical practice for patients with AL amyloidosis remains unclear due to its rarity and heterogeneity. **Aims.** The aims of this investigation are to convey a practical picture of the disease, its response to treatments, and outcomes in terms of organ function and survival of patients with AL amyloidosis in Hokkaido, Japan's northern islands, regardless of age, disease severity, or treatments. **Methods.** Hokkaido Hematology Study Group conducted a questionnaire survey about clinical practice for patients with AL amyloidosis diagnosed from January 1, 2006 to December 31, 2010. All patients were biopsy-proven and consecutively accumulated. Patients received treatments in local hematology centers, and regimens and doses were at the discretion of treating physicians. Organ involvement and treatment response were evaluated according to a consensus opinion from the 10th international symposium on amyloid and amyloidosis. **Results.** Data from 39 patients were accumulated from eleven secondary or tertiary referral centers for hematologic diseases in Hokkaido. Forty-nine percent were male and median age at presentation was 67 years (range 32 to 82). Median time from awareness of subjective symptom to the first visit to clinic was 39 days (maximum 7 years). Renal (49%) and cardiac (31%) presentations were predominant, followed by symptoms from gastrointestinal tract or neurological involvement. Most (89%) of the patients with renal symptoms have already suffered from nephrotic syndrome. Median time from the first visit of patients to the diagnosis was 64 days (range 7 to 367 days). Involvement of 1 organ was present in 9 patients, 2 organs were involved in 10, and 3 or more organs in the remaining 20. Distribution of organ involvement was as follows: kidney, 77%; gastrointestinal tract, 74%; heart, 49%; peripheral neuropathy, 23%; and liver, 15%. First-line treatment was with traditional regimen (like MP or VAD) in 8 cases, with novel agents (bortezomib or thalidomide) in 14. Fourteen patients received a high-dose melphalan with autologous stem cell transplantation (ASCT) as first line treatment. Hematologic CR observed in 11 (32%) cases, PR in 3 (9%), SD in 20 (59%); organ response was observed in 5 (16%) cases. At the time of analysis, median survival time from diagnosis was 524 days (range 51-1536 days). Cases of death include heart failure, infection, and renal failure; 13 patients have died within one year from diagnosis. Cardiac involvement, serum brain natriuretic peptide >200 pg/ml, and uric acid >7. 0 mg/dl were risk factors for death. Patient receiving ASCT with 200 mg/m^2 of melphalan pretreatment significantly prolonged survival. **Conclusions.** This study has revealed present status of clinical practice for patients with AL amyloidosis in designated area. The inclusion of most patients, regardless of disease severity, indicates a persistently poor prognosis among a substantial proportion of patients in a practical setting, and highlights the unmet need for improved diagnosis and treatment strategies.

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VALUE OF THE FREE LIGHT CHAIN ANALYSIS IN THE CLINICAL EVALUATION OF RESPONSE IN MULTIPLE MYELOMA PATIENTS RECEIVING ANTI-MYELOMA THERAPY

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Introduction. More than 95 % of patients with multiple myeloma (MM) have an increase of either κ or λ free light chains (FLC) in serum, or an abnormal κ/λ-ratio (rFLC). Since the FLC have a very short serum half-life of 2-6 hours, compared with the serum half-life of the intact immunoglobulin, being days to weeks, we would expect that measuring FLC could give us an advantage in the response evaluation of the therapeutic effects of a given anti-myeloma treatment. **Aims.** Assess the time to 50 % reduction of serum involved FLC ("clini-

cal"half-life, $t_{½}$) in MM patients receiving anti-myeloma therapy, and to determine if measurements of involved FLC (iFLC) provide faster, yet reliable, information on the effectiveness of a given anti-myeloma treatment. **Methods.** We measured the levels of iFLC in 18 patients with symptomatic MM before treatment, 1, 2, 3, 4, and 5 week days after the first anti-myeloma treatment, and 2, 3, and 6 weeks after. Informed consent was obtained. The patients were evaluated for response according to the international uniform response criteria. Serum half-life were calculated using the exponential regression model, based on the formula $y = b * a^x$. A significant reduction in FLC was defined as $\sqrt{2} * 1.96 * \sqrt{CVtotal^2}$. The patients were treated with bortezomib-, thalidomide-, or alkylator-based chemotherapy. **Results.** Seven (39 %) patients achieved VGPR or better, 4 (22 %) PR, 6 (33 %) SD, and 1 patient was not evaluable (died in first treatment). Four patients had iFLC below 100 mg/L, and only one of those showed a significant reduction in iFLC, while 3 had no reduction and therefore not evaluable $t_{½}$. Fifteen patients (83 %) had an initial significant reduction in iFLC. The half-life of iFLC was 4. 8 (2. 2 - 9. 6) days for patients achieving ≥ VGPR, 7. 2 (1. 8 - 18) days for patients achieving PR, and 4. 1 (2. 7 - 5. 6) days for patients with SD. The patients achieving ≥ VGPR all had an early response and a continued significant reduction of iFLC between second and third cycle of treatment. **Summary and Conclusions.** The serum half-life is very different (1. 8 - 18 days) in MM patients but not predictive of final response. Surprisingly, all patients with pre-treatment iFLC levels >100 mg/L showed early significant reduction, even patients with SD. To predict achievement of good response it is important to observe a continued decline in iFLC during the following treatment cycles. Our sampling is continuing and we will be able to present data on a larger cohort at the meeting.

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PRELIMINARY EXPERIENCE OF THE SPANISH COMPASSIONATE USE REGISTRY OF BENDAMUSTINE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA (MM R/R)

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Background. Bendamustine is a dual alkylating agent with demonstrated efficacy in de novo MM and MM R/R. We present preliminary results from the experience of Spanish compassionate use registry of this agent in different treatment schedules of MM R/R, promoted by the GEM / PETHEMA Group (Study Benda-MMRR-11). This study has been approved by local ethical committee, CEIC, HUP, Madrid, Spain. **Patients and Methods.** 20 patients, 11 males and 9 females, with advanced MM R/R after several lines of previous treatment received a schedule containing Bendamustine. Median age was 63 (35-86), with a median of 6 previous rescue lines (1-9). Bendamustine dose used ranged between 90 and 100 mg/m2 iv on days 1 and 2 for each 28 days cycle. The median of cycles was 4 (1-8). The combinations used were: Bendamustine + Bortezomib + dexamethasone in 5 patients, Bendamustine + Dexamethasone in 5 patients, Bendamustine + Thalidomide + Prednisone in 9 cases and Bendamustine + Prednisone in one case. **Results.** In the 18 evaluable patients, overall response rate (ORR) was 49. 9%, with partial response (PR) of 33. 3%, complete response (CR) of 11. 1%. Minimal response (MR) was of 5. 5%, and stable disease (SD) was of 5. 5%. Progression was documented in 44. 4% of patients. In general, treatment was well tolerated; the most common adverse effect was hematological toxicity. We observed, grade 3-4 neutropenia in 38%, grade 3-4 thrombocytopenia in 33. 3% and grade 3-4 anemia in 22%. Among other toxicities noted infections in 16% of patients. **Summary.** Treatment with Bendamustine can be a therapeutic alternative in patients with MM R/R. Our results are consistent with published data in larger series of patients. More experience is needed with this agent, find the best combination and assess its grade of efficacy in earlier stages of myeloma evolution.

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BENDAMUSTINE COMBINATION THERAPY IS ASSOCIATED WITH POOR OUTCOME IN MYELOMA PATIENTS WITH ADVERSE CYTOGENETICS WHO RELAPSED FOLLOWING A PROTEASOME INHIBITOR AND ARE REFRACTORY OR INTOLERANT TO IMIDS

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Background. Treatment options for MM patients who relapse following bortezomib and IMIDs remain limited, with a median overall survival of 9 months (Kumar et al, Leukemia 2012). At least part of this is due to the accumulation of adverse cytogenetic features with disease progression such that end stage patients frequently exhibit poor risk features. Therefore therapies must have activity in such patients to be of benefit. Bendamustine is a cytotoxic alkylating agent with pre-clinical activity in chemotherapy resistant MM. It has been increasingly used in the relapse/ refractory setting however its activity in high risk cytogenetic MM has not yet been determined. **Aims.** To determine the efficacy of bendamustine combination therapy in patients with relapsed and/ or refractory myeloma particularly those with adverse cytogenetic features. **Methods.** A retrospective single centre study of 13 patients with relapsed MM that received bendamustine 60mg/m^2 on days 1, 8, 15 of a 28 day cycle. 4 patients received corticosteroids whilst 9 received thalidomide (up to 100mg) in addition to corticosteroids. Responses were assessed by IMW response criteria and progression free survival (PFS), and overall survival (OS) assessed according to IMW consensus criteria. Interphase FISH was performed on CD138 selected cells. **Results.** Median age was 62 years (range 46-80) and patients had received a median of 4 prior lines of therapy (range 3-6). All patients received prior bortezomib and twelve patients (92%) received prior IMID (10 prior lenalidomide). Nine patients (69%) had disease progression on their last line of therapy whilst four (31%) had stable disease. Cytogenetic information was available on 10 patients (77%) of whom four (40%) had adverse cytogenetic features including 17 p deletion (n=1), t(4:14) (n=1) and chromosome 1 abnormalities (n=4). Patients received a median of 3 treatment cycles (range 1-6). Grade 3/4 treatment emergent toxicity was as follows: neutropenia (n=5), anaemia (n=1), thrombocytopenia (n=1) with ≥grade 2 infection in 5 patients. Overall response rate (≥PR) was 23% (PR 3; 23%, SD 3; 23%, PD 7; 54%) with a clinical benefit rate (≥SD) of 46%. After a median follow up of 3. 9 months (range 1. 0-11. 0), the median PFS was 3. 6 months. For those with adverse cytogenetics (n=4) the median PFS was 2. 7 months, compared to median PFS not yet reached for standard risk disease. The addition of thalidomide made no difference to response or PFS. Two (15%) died of progressive disease, 1 (8%) died of unrelated causes, and 9 (70%) remain alive (1 patient lost to follow up). The median OS has not yet been reached. **Conclusions.** For MM patients who have relapsed following bortezomib and predominantly refractory to IMID, the clinical benefit rate (≥SD) for bendamustine combination was 46% with no benefit with addition of thalidomide. Patients with adverse cytogenetic features had extremely poor outcomes (median PFS of 2. 7 months, compared to 3. 6 months whole group). Although a limited study, this suggests that such patients would not benefit from this approach and should be considered for combinations with other novel agents such as proteasome or HDAC inhibitors.

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ASSESSMENT OF BONE METABOLISM IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is a plasma cell malignancy characterized by bone destruction with activated osteoclast (OC) and suppressed osteoblast (OB), which is sometimes associated with hypercalcemia. Increased levels of bone resorption marker such as urinary NTx and CTX-1 were observed in MM patients with osteolysis. Tartrate-resistant acid phospatase 5b (TRACP-5b) is one of bone resortion markers, which has recently been introduced. Since TRACP-5b, bone formation markers procollagen type I N-terminal propeptide (PINP) and bone alkaline phosphatase (BAP) are not affected by renal function, they may be suitable for the assessment of bone metabolism in MM. **Aims.** The aim of this study is to assess bone metabolism by TRACP-5b, PINP, BAP, and osteocalcin (OC) in untreated MM patients with progressive osteolysis. **Methods.** We measured serum concentrations of TRACP-5b by EIA (normal range are 170-590 and 120-420 mU/dL in men and women, respectively), PINP by double antibody radioimmunoassay (normal range are 19. 0-83. 5, 14. 9-68. 8, and 27. 0-109. 3 µg/L in men, premenopausal women, and postmenopausal women, respectively) and BAP by CLEIA (normal range are 3. 7-20. 9, 2. 9-14. 5, and 3. 8-22. 6 µg/L in men, premenopausal women, and postmenopausal women, respectively), and OC by IRMA (normal range is 2. 5-13. 0 ng/mL) in ten untreat-

ed MM patients (4 men, 6 women; age range, 49-82 years; mean age, 69 years) with osteolysis revealed by computer tomography. **Results.** The mean serum concentrations of Ca, Cr, TRACP-5b, PINP, BAP, and OC were 10. 8 mg/dl, 1. 49 mg/dl, 585. 1 mU/dL, 85. 2 µg/L, 28. 5 µg/L, and 8. 0 ng/mL respectively. Serum TRACP-5b levels were within normal range in 6 patients and higher than the normal range in the remaining 4 patients. In a simple regression analysis, TRACP-5b levels had a weak correlation with PINP levels (r=0. 50, p=0. 14), although they had no correlation with BAP (r=0. 09, p=0. 79) and OC (r=0. 19, p=0. 59). Serum PINP levels were higher than the normal range in 5 out of 10 patients, lower than the normal range in 2 patients, and were within the normal range in 3 patients. Mean serum Ca level was 10. 9 mg/dl in patients with high PINP levels whereas it was 8. 7mg/dl in other patients (p=0. 08). A weak correlation was found between basal levels of serum Ca and PINP (r=0. 36, p=0. 30), although there was no correlation between basal levels of Ca and BAP as well as TRACP-5b (r=0. 11, p=0. 77), suggesting a possible impairment of bone mineralization in patients with high PINP levels. **Conclusions.** TRACP-5b levels had a weak correlation with PINP but not with BAP and OC. We unexpectedly found the marked diversity of serum PINP levels in MM patients. The patients with high PINP levels had a tendency of hypercalcemia.

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VELCADE-DEXAMETASONE CICLES UNTIL THE BEST RESPONSE FOLLOW BY AUTOLOGOUS STEM-CELL TRANSPLANTATION AS FIRST LINE OF THERAPY IN YOUNGER MULTIPLE MYELOMA PATIENTS

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Purpose. Analysis of response to Velcade-Dexametasone treatment until maximum response before autologous stem-cell transplantation (ASCT) in patients newly diagnosed of Multiple Myeloma (MM). Protocol: Induction therapy: Velcade was administrated at dose of 1,3 mg on days 1,4, 8,11 of each cycle. Dexametasone at dose of 40 mg/m^2 on days 1-4, 9-12 and 17-20 of each cycle. Response was evaluated in the first day of each cycle performing proteinograme, inmunofixation in blood and urine. We determine maximum response when monoclonal component remain estable for two consecutive cycles. Mobilization: was performed with G-CSF. Melphalan at dose of 200 mg/m^2 was used as conditioning regimen in all the patients. No post-trasplant chemotherapy was permitted until relpse or progression. **Patients.** 21 consecutive patients of Hospitals of Extremadura from March 2009 to March of 2011. Men/Women: 8/13. Median age was 52,3 (41,7-68,9). Durie-Salmon estage was: IA: 6 patients, IIA: 4. IIIA: 7, IIIB: 4. IgG: 7 (33%), IgA: 6(28,5%), Bence-Jones 7(33%), non-secretor 1 (4,76%). Karyotype: Normal: 13 (61%); del 17p: 2(9,5%), t(11,14): 1, t(4,14): 1, add 14+t(11,14). 1, t(4;14): 1. Non-avaible: 2. Three patients presented plasmocytomas at diagnosis. Beta-2-microglobulin was 3,5 (1,8-12,07). Number of cycles administrated was 4(3-9). Median follow-up is 22,4 months (12,566-38,43 months). The median follow-up from ASCT was 9,6 (7,2-22). No-one patient died during induction therapy and during ASCT. The median of aphaeresis performed by patient was 1 (1-4) and the median CD34 cells obtained were 3,6x10^9/L (2,5-7,3x10^9/L). The number of complete responses after induction therapy were 7 (33%); partial response 12 (57%), and failure 2(9,5%). The number of patients in complete response three months after ASCT were 14 (66%). No patient has death. Six patients have relapsed and Five are in treatment with Lenalidomide-Dexametasone at a median time of 16 months after ASCT. **Conclusions.** Increasing the number of cycles of VD before ASCT is a feasible strategy to treatment of new diagnosed MM. The rates of CR and PR are comparable to three drugs scheme as Velcade-Thalidomide-Dexametasone (VTD).

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LENALIDOMIDE AS RESCUE THERAPY IN MULTIPLE MYELOMA: A SINGLE-CENTRE EXPERIENCE

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Background. In recent years, new first line therapeutic strategies induce a high percentage of remissions in multiple myeloma (MM) patients, but, eventually, a great amount of patients relapses and requires further treatment. **Aims.** retrospective evaluation of response to lenalidomide and related toxicity in refractory/relapsed patients in a single centre real-life setting, outside of clinical trials. **Methods.** From October 2007 until November 2011, 47 MM patients have been included: 27 males, 20 females; median age 74 years (range, 50-89); 33 IgG, 6 IgA, 5 light chain, 3 non secretory. Eight patients (17%) had renal failure. Median time from diagnosis was 41 months (range 3-238 months) and 15 patients had received autologous bone marrow transplantation (ABMT) as first line approach. In 20 patients we used lenalidomide as second line (43%), in 14 patients as third line (30%), in 8 patients as fourth line (17%), in 3 patients as fifth line (6%) and in 1 patients as sixth and seventh line (2% respectively). Several combination were used: 2 CPR, 1 RAD, 1 MPR. Median number of cycles was 7 (range, 2-37), 13 patients are in active treatment; treatment was continued until progression. **Results.** According to IMWG criteria, 28patients (60%) achieved a response [1 (2%) CR, 10 (21%) VGPR, 17 (36%) (PR)], while 19 patients (40%) had a stable/progressive disease (SD/PR). Median PFS at 15 months was 60% and OS from the onset of lenalidomide was 47% at 24 moths. With a median follow up of 14 months (range, 4-44), 32 patients had progressed (68%) and 19 of them (40%) have deceased. Average response was 5 month (range, 1-27). No differences in obtaining response or in length of PFS due to age (less than vs greater than 65) or response (VGPR/CR vs PR) were found, while PFS is significantly better considering number of previous line of therapy (1-3 lines vs more than 3 lines;21 m. vs9 m) (p less than0,05). No difference has been shown in OS in the different groups. Furthermore we find a difference PFS in patients obtaining even only a partial response vs a stable disease (22 m. vs6 m; p 0,001). No difference has been shown in OS of the two groups. Grade 3/4 adverse event included 10 patients with neutropenia (21%), 2 patients with thrombocytopenia and fatigue (4%), 1 patient with rash (2%), 1 patient with lymphopenia (2%), cardiac failure, pneumonia (3%). Seven patients (21%) delayed or discontinued therapy due to toxicity. 11 patients (23%) had presented neuropathy during lenalidomide treatment. No secondary primary malignancies were found during lenalidomide treatment (only 5 patients (11%) were treated with more/equal 24cycles). **Conclusions.** This retrospective analysis shows effectiveness and safety of lenalidomide when used in routine clinical practice similar to those reported in clinical trials. In addiction it demonstrates the importance of obtaining even a partial response against a stable disease, at least in this setting of patients who are continuously treated until progression.

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IMPROVING RESPONSE RATE IN ELDERLY PATIENTS AFFECTED BY MULTIPLE MYELOMA USING LENALIDOMIDE AS PROLONGED THERAPY

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Background. lenalidomide is a new immunomodulatory drug with a dual mechanism of action: tumoricidal effect and immunomodulatory effect. It represents an important treatment option for multiple myeloma patient either as first line therapy, either in resistant/refractory disease or consolidation/maintenance therapy. In this way lenalidomide increases the available treatment options. **Aims.** In our Department, lenalidomide was administered in elderly patients affected by resistant/relapsing myeloma or as consolidation/maintenance therapy in patients with stable disease (partial remission) after induction therapy or more lines of chemotherapy. **Methods.** We treated 44 patients (23 M and 21 F) with median age of 70 years (range 66-81). We have evaluated 33 patients with a median follow up of 24 months. These patients were treated with lenalidomide at variable doses (5-25 mg/die p.o., according to tolerability of each patient, for 21 days every 28 days) alone as continuous treatment. We used enoxaparin for prophylaxis of venous thromboembolisms. Clinical restaging was performed after three, six and twelve courses of therapy. **Results.** At present we have observed in 24/33 patients a good impact on monoclonal component. 15 out of 33 patients previously in PR shifted to a better responce (5 to nCR or CR and 10 to VGPR). In the other 7, previously in stable disease, we observed, after a median of 8 courses of lenalidomide, a shift to a PR. Therapy was well tolerated in all patients and we didn't observed any significant adverse events. **Conclusions.** Lenalidomide as prolonged therapy can have an established role in a very bad subset of patients as the previously treated elderly myeloma patients. Our data demonstrate that lenalidomide improves response rate in these patients without causing severe complications.

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TREATMENT OF BENCE-JONES MULTIPLE MYELOMA WITH CHEMOTHERAPY WITH/WITHOUT STEM CELL TRANSPLANTATION-SINGLE CENTRE EXPERIENCE

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Background. Bence-Jones meyloma multiplex is a progressive disease characterized by excesssive numbers of abnormal plasma cells in the bone marrow and overproduction of incomplete immunoglobulins, containing only the light chain portion of the immunoglobulini. This type of myeloma occurs 15-20%. The median overall survival is approximately 4 years. Patient outcome in Bence-Jones myeloma has been remarkably improved due to the use of combination therapies including chemotherapy and stem cell transplantation. High-dose melphalan with autologus stem cell support has been an integral part of myeloma therapy for more than 25 years. **Aims.** A retrospective analysis of outcome of treatment Bence-Jones myeloma. **Patients and Methods.** Since 1995. until 2011. we were treated 54 pts (37 men and 17 female), average age 56 years (range 36-85). According to the clinical stage, patients were divided as follows: 3 pts ICS, 13 pts IICS, 38 pts IIICS. Regarding ISS score, the group included: ISS1 16 pts, ISS2 9 pts, ISS3 29 pts. Renal insufficiency was present in 30 pts. High risik pts was defined by the presence of following factors: b2M >5,5 mg/l, albumine<3,5gr/dl, CRP >6, high lactat dehydrogenase, renal failure, stage III. Patients treated with induction, consolidation and maintenance therapy. Conventional induction treatments were applied as following regimens: VAD (40), MP (9), CTD (1), PAD(2) and TAD (2). **Results.** Conventional chemiotherapy introductory clinical response was achieved in 35pts (65%) (MR-4 pts, PR- 14 pts, VGPR- 17 pts), while in 19 pts (35%) established disease was resistant. Transplantation had been done with 29 pts (54%), while 25 pts (46%) were treated with conventional chemotherapy adjusted to the vital age and comorbidity. In the group of pts with transplantation done tandem had been carried out with 9 pts and secondary SCT had been done in 4 relapsed pts. With 1 pts with tandem SCT allogenic(singen) SCT had been done. TRM is 3,4%. Maintenance therapy with Thalidomid had been done in 25 pts for 4-38 months. Impact of high risk factors on outcome/TTP/OS was of no significance, but the transplanted patients had significantly longer TTP (mediana 8 months vs 6 months, p=o,o11) and longer OS (mediana 42 months vs 20 months,p=0,0003). 20 pts (37%) of treated pts are living, while 34 pts (63%) died. Univariate log. regres. analysis showed that non-transplant patients are 10,41 times more likely to terminate lethal compared to transplant patients (RR 10,41(95%C. I. 43,47-2,52),p<0,001). **Conclusions.** Our study showed ASCT is a more effective method of treatment of patients with BenceJones myeloma compared to the conventional chemotherapy, but the results are still unsatisfactory. One of the major efforts to improve the results of intensive therapy and ASCT involves the integration of novel agents (proteasome inhibitors and immunomodulatory drugs) into the transplantation sequence.

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IGM MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (IGM MGUS): BETWEEN THE ANALYTIC FINDING AND B-CELL MALIGNANCIES

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Background. MGUS is common in the general adult population aged 50-60 years or older, the IgM subtype is the less common and the incidence of lymphoproliferative disorders associated with a monoclonal IgM component in the serum is a very rare disease, 1-2% of hematological malignancies. A personal history of the precursor condition IgM MGUS is associated with a risk of developing lymphoplasmacytic lymphoma/Waldenström´s macroglobulinemia LPL/WM and an increased incidence of second neoplasm has been reported in lymphoproliferative disorders. **Aims.** To describe demographic, clinical and outcomes features of our serie of patients with IgM MGUS. **Methods.** A review of medical records of patients diagnosed or followed by IgM MGUS between january 1990 and december2010 ina single center was performed. Data on clinical, biochemical, inmunological and cytogenetics features, therapy, second malignancies and the mortality were obtained. Recently we have incorporated the Hevylite measures IgMkappa and IgMlambda, separately, and apply to determine the IgM M-spike in our cohort. **Results.** A total of 27 patients were registered (M/F: 15/12), the median age was 67 (46-86) years. Diagnosis of IgM MGUS 11 / 41% and LPL/WM 16 / 59%: symptomatic 5 / 31% and smoldering 11 / 69%. At the time of diagnosis the patients had developed enlarged spleen (40%), visual disturbances (40%), neuropathy (20%), hyperviscosity symptoms (20%) and hepatomegaly (20%). We have analyzed the ratio IgMkappa/IgM-lambda as biomarker of malignancy significant. The incidence of second neoplasia was 15% and B-cell malignancies in first-degree relatives 8%. High b2-microglobulin (93%), increased sedimentation rate (74%), anemia (30%) and low platelet count (11%) were the most common analytical features. The median concentration of M-spike was 1. 43 mg/dL (0. 32 - 3. 49), 65% kappa subtype, proteinuria was not detected. The median bone marrow lymphoplasmacytic infiltration was 31. 5% (12. 6-82. 2), usually interstitial, and mast cells was seen in 35% of patient. Typical inmunophenotype (CD5- CD10-, CD19+, CD20+, CD23-) by flow cytometry in 52% of the patients and cytogenetic abnormalities in 5 / 19%: del(6q21): 60%, t(14q32): 40%, t(18q21): 20% and t(13q14): 20%. There were progression in 3 cases (to symptomatic LPL/WM, NHL's large cell-B brain) after an average follow-up of 12 years. The first-line therapeutic approachs was needed in 6 (22%) cases, with alkylating agent-based regimens 4 / 66%, rituximab 1/17% and R-COP 1 /17%, a partial response in all cases was obtained and relapse/progression in 4 / 66% cases. Plasmapheresis in one case by hyperviscosity syndrome. The median survival free of progression: 20 months Mortality: 4 / 15% patients: two by infection, one by progression and one by bleeding. **Conclusions.** In our experience, the discovery of IgM MGUS is not synonymous of malignancies, have been frecuently indolent (81%) and slow progression, 13. 6% to the 12 years of the diagnosis. We highlight the development of second malignancies in 15% of cases. Despite 59% of the cases met criteria of LPL/ MW only 22% received chemotherapy treatment. Future studies are need to define molecular markers that distinguish low - and high-risk precursors patients.

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REMISSION DURATION AND QUALITY OF RESPONSE IMPROVEMENT IN ADULT MULTIPLE MYELOMA PATIENTS WITH BORTEZOMIB MAINTENANCE POST AUTOLOGOUS STEM CELL TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

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Background. In the past decade single or tandem ASCT was considered the standard treatment in adult MM. However ASCT demonstrated to be not able to prevent disease progression. Thus in these years a post-ASCT maintenance based on bortezomib, thalidomide or more recently lenalidomide is becoming the consolidating approach in the aim to prolong and/or to improve post ASCT response. **Aims.** In December 2007 asingle center study has been activated to define the impact of Bortezomib single agent maintenance for 24 months a) on time to progression (TTP) and Time to Next Treatment (TNT) b) the feasibility and toxicity of this treatment. **Methods.** Between November 2006 to June 2009 14 MM pts- underwent single (2) or tandem (12) ASCT received Bortezomib maintenance (BG). Of these 9 were males and 5 females- median age 58 y (range 29-67y)- At MM diagnosis 9 were IgG (7 k, 2 λ), 3 IgA (1 k, 2 λ), 2 micromolecular (1k, 1 λ). As stage11 pts were D-S ≥III (13 A, 1B) and4 pts ISS ≥III, respectively. Baseline cytogenetic showed 13 q- in 5/13 pts studied. We considered as control group 13 pts-5 males and 8 females- median age 60(range 51-64 y)-underwent single (7) or tandem (6) ASCT. . Maintenance schedule consisted of Bortezomib as single agent given at dosage 1. 5 mg every 15 days until progression. Response was evaluated according to the International Myeloma Working Group criteria. Peripheral neuropathy (PN) was monitored before maintenance start, then every 3 mo by neurophysiologic motor and sensory conducting tests. **Results.** In the BG maintenance was started in a median time of 3. 1 mo (range 1. 7 -13. 7), from ASCT,8 ptswere in CR orVGPR, 6 PR. The overall response rate was 72%, with 57% CR+VGPR. As of February 2012, after a median maintenance time of 20 mo (range 4. 1 -24); 10/14 pts are alive. 7 (50%) in CR, 1 was lost to follow-up, 2 with progressive disease. It is surprising that FISH analysis in 4/5 progressive-relapsed disease during maintenance had 13q- abnormality at diagnosis. Since not all pts underwent 2nd ASCT, TTP and TNT was evaluated from 1st ASCT. In the BG median TTP is 32 mo, whereas in the CG median TTP is 12 mo (p<0. 005). Median TNT in BG is 34 mo whereas in the CG median TNT is 13 mo (p< 0. 005). At median follow up of 62 mo (38-71) median OS has not yet been reached in BG, In CG at median follow up of 96 mo (55-119) median OS is 58 mo (p= n. s.). Finally, none of pts in the BG experienced grade 3 or 4 haematologic toxicity and/or PN requiring dose reduction or drug discontinuation . **Summary and Conclusions.** the prolonged follow up confirms that Bortezomib-single agent maintenance in post-ASCT is active and safe to prolong TTP, TNT and able to improve response quality.

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CYCLOPHOSPHAMIDE THALIDOMIDE DEXAMETHASONE (CTD) AS FIRST-LINE THERAPY IN MULTIPLE MYELOMA. EXPERIENCE IN A HOSPITAL IN LIMA, PERU

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Multiple myeloma is a common disease in our hospital presenting 40 to 50 new cases a year. We present a retrospective study of 41 patients undergoing chemotherapy CTD as first-line regimen in the Rebagliati Hospital - EsSalud of Lima, Peru, between May 2008 and December 2011. The age of patients was 31-76 years (mean: 41 y). Men 24 and women 17. Debut ISS score: I (8), II (14), III (10). The responses were: CR (8), VGPR (7), PR (17), SD (1) and PD (2). CR + VGPR: 42% (15), CR + VGPR + PR: 91% (32). Time to response 3-12 months (mean: 5 m). Progression-free survival (PFS): mean 14. 8 months (1. 4-39. 6 m). 15 patients underwent autologous BMT. CTD is an effective and inexpensive treatment of MM for countries with limited economic resources.

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CYTOKINE PROFILES IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTEMIA: CLINICAL IMPLICATIONS

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The most frequent complications of Polycythemia vera (PV) and Essential thrombocythemia (ET) are thrombosis and bleeding risks that influence decision of a specific therapy. One mechanism involved cytokines that put forward modulate haemostasis and thereby favour thrombotic events. The hypothesis of a clinical impact of plasma cytokines levels is reinforced by reports about the mechanism of JAK2 inhibitors on constitutional symptoms, consequence of the down-regulation of some cytokines levels. In this context we have initiated a comparative study of plasma cytokine expression profiles as a function of vascular complications and JAK2V617F status of PV and ET patients. From the plasma of seventeen patients diagnosed with PV and twenty one patients affected by ET, cytokine measurements were carried out using a multiplex cytokine assay. Cytokine chosen were pro-inflammatory cytokines: IL-1β, IL-6, IL-8, IL-12(p70), TNFα and IFNγ, anti-inflammatory cytokines: IL-4, IL-10, the MCP-1 chemokine and growth factors: GM-CSF, PDGF-BB, VEGF and HGF. Twenty two of these patients (10 PV and 12 ET) have experienced at least one thrombo- hemorrhagic manifestations. Comparing cytokine profiles between the group with and without vascular complications, no significant difference was observed except for IL-12(p70) for which the difference was hardly significant (p=0. 047). However, the subgroup of PV patients displayed a significant difference for IL-12(p70) (p=0. 025) and for GM-CSF (p=0. 040). No such difference was observed with the group of ET patients. We have also compared cytokine profile between ET and PV patients and we have clearly discerned different cytokine profiles with a statistical significance for IL-4 (p=0. 006), IL-8 (p=0. 003), GM-CSF (p=0. 026), IFN-γ (p=0. 007), MCP-1 (p=0. 009), PDGF-BB (p=0. 015) and VEGF (p=0. 017). Interestingly, all cytokine concentrations were higher in ET patients compared to PV ones. The same differences of cytokine profiles except for PDGF-BB were obtained between PV and ET patients who have had a vascular complication. Regarding JAK2 mutational status, we observed very few differences. Only TFNα (p=0. 042) and PDGF-BB (p=0. 019) were specifically impacted by JAK2 V617F status in PV and ET, respectively. In conclusion, our results suggest that PV and ET patients display significantly different plasma cytokine levels, although the link with JAK2 V617F only concerns TNFα for the PV group and PDGF-BB for the ET one. We also observed that IL12 (p70) and GM-CSF should be useful biomarkers of the vascular risk. Altogether these data suggest that cytokine profiles may impact significantly the phenotypic characteristics of PV or ET and they could influence the rational for JAK2 inhibitor prescription.

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ANGIOGENIC FACTORS EXPRESSION IN BONE MARROW AND PERIPHERAL BLOOD OF MYELOPROLIFERATIVE NEOPLASMS

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The microvessel density of bone marrow is increased in myeloproliferative neoplasm (MPN), associated with an enhanced expression of vascular endothelial growth factor (VEGF) as an inducer of angiogenesis. The nitric oxide (NO) mediated VEGF expression as well as the VEGF-mediated NO production by endothelial NO synthase (eNOS) are regulated by hypoxia inducible factor (HIF)-1 activity, depending upon the amount of produced NO. The aim of the study is to investigate the expression of major angiogenic molecules, and their correlation, in bone marrow and peripheral blood of MPNs. The bone marrow and granulocytes are examined for the expression of angiogenic factors VEGF, HIF-1 alpha, and eNOS using immunohistochemistry and Western blot. Microvessel density is assessed by immunostaining with CD34 antibodies. A somatic point mutation JAK2V617F is detected in 93% of polycythemia vera (PV), 64% of primary myelofibrosis (PMF) and 59% of essential thrombocythemia (ET) patients, out of total 95 MPN patients. Microvessel density is significantly higher in MPNs than in controls, being highest in PMF (35-37%), followed by ET and PV. The presence of JAK2V617F mutation augments the percentage of CD34-positive cells in MPNs. The percentage of VEGF-positive cells is increased in the bone marrow of PMF patients (6. 8%), more prominently than in other MPNs. The increase of V617F allele burden did not change significantly VEGF percentage in PV, although demonstrates elevation in ET patients from 4. 3% to 5. 6%. VEGF protein levels are also increased in granulocytes of MPN patients and correlate with JAK2V617F allele burden in PMF patients (up to 2. 5 fold). In contrast, eNOS protein levels are decreased in granulocytes of MPN patients, however less in patients with high JAK2V617F allele burden. The most prominent eNOS protein level (2. 7 fold) is observed in JAK2V617F homozygous form of PV. Low HIF-1 alpha protein levels are also more prominent in granulocytes of MPN patients with JAK2V617F mutation, with the highest level in PMF. The percentage of HIF-1 alpha-positive cells (3-8%) is also increased in the bone marrow of MPN patients. According to presented results, the microvessel density is increased in MPN with high JAK2V617F allele burden and angiogenic factors level in the bone marrow are in correlation with their levels in peripheral blood granulocytes. Moreover, VEGF is also enhanced in MPNs in accordance with HIF-1 alpha and opposite to eNOS. Therefore, the positive feedback between VEGF and eNOS is inversely regulated by HIF-1 alpha in MPNs.

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GALECTIN-1 AND GALECTIN-3 EXPRESSION IN MYELOPROLIFERATIVE NEOPLASMS PATIENTS

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Background. Polycythemia vera (PV), Essential thrombocythemia (ET) and Primary Mielofibrosis (PMF) are clonal haematopoietic stem cell malignances characterized by an accumulation of mature myeloid cells in bone marrow and peripheral blood in the absence of a definable stimulus. A specific point mutation in the Janus kinase 2 gene (JAK2V617F) has been identified in more than 90% of patients with PV and in 50%-60% of ET and PMF patients. However many aspects of pathogenesis such as how a single mutation can lead to three different diseases and the description of specifics diagnostic markers for each disorder remains unclear. Deregulation of apoptotic machinery may have a role in MPNs physiopathology. The better understanding of apoptosis and its potential modulation by galectin-1 and 3 in PV, ET and PMF patients' cells might unveil novel therapy targets. **Aims.** 1) To quantify *LGALS-1* and *LGALS-3* expression in $CD34^+$ hematopoietic stem cells and in leukocytes from PV, TE and PMF patients and controls; 2) To correlate the gene expression results of *LGALS-1* and *LGALS-3* with apoptosis-related genes from bcl-2 family. **Subjects and Methods.** Bone marrow (BM) $CD34^+$ cells and peripheral blood (PB) leukocytes from 26 PV patients (9 males and 17 females with a mean age of 62y), 26 ET patients (5males and 21 females with a mean age of 61. 1y) and 14 PMF patients (12 males and 2 females with a mean age of 63. 7y) were analyzed. The control group comprised 15 BM donors (9 males and 6 females, m=28. 5y) and 46 PB donors (16 males and 30 females, m=57. 8y). $CD34^+$ cells were enriched by using immunomagnetic isolation and peripheral leukocytes were obtained by Haes-Sterilmethod. Total RNA was extracted according to Trizol□' method and High Capacity® Kit was used to synthesize cDNA. The expression of *galectin-1* and *3* and apoptosis-related genes was performed by real time PCR. The gene expression results were given as $2^{-\text{Delta Delta Ct}}$. Statistical analyses were carried out by

Mann-Whitney and Spearman tests. **Results.** *GALECTIN-1* levels were decreased in ET (median=0. 66) and PMF (0. 73) leukocytes and in PV (0. 62) $CD34^+$ BM cells when compared to controls (0. 96; 0. 96; respectively) (p=0. 042; p=0. 009; p=0,018; respectively). *GALECTIN-3* levels were decreased in ET leukocytes (0. 081) in comparison to controls (0. 87) (p<0. 0001). ET $CD34^+$ cells (1. 18) showed higher levels of *GAL-1* than PV (m=0. 62) and PMF (0. 81) cells (p=0. 001; p=0. 002; respectively). ET leukocytes (0. 081) showed lower levels of *GAL-3* than PV (1. 23) and PMF (0. 58) (p<0. 0001; p=0. 0001; respectively). In addition, we found a positive correlation between *GAL-3* expression and anti-apoptotic gene *MCL-1* in leukocytes (r=0. 39; p=0. 02) and in $CD34^+$ cells (r= 0. 41; p= 0. 01). **Conclusions.** *GALECTIN-3* seems to be linked to *MCL-1* expression, an anti-apoptotic gene. We highlight the differences in *GAL-1* and *GAL-3* expression in ET compared with PV and PMF. The results indicate the involvement of these lectins in the MPN physiopathology.

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TRANSCRIPTOMIC ANALYSIS OF NATURAL KILLER CELLS IN POLYCYTHEMIA VERA

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Background. The Natural Killer cells (NK) cells are innate immunity with the ability to lyse tumor cells. Abnormalities of their cytotoxic capacities have been described in several hematologic malignancies. The polycythemia vera is a myeloproliferative disease characterized by the almost constant presence of a mutation of JAK2. **Aims.** The aim of our study is to determine whether this mutation may participate in NK function abnormalities that we previously described (Sanchez et al., Manuscript in preparation) in polycythemia vera. **Methods.** We chose an approach by transcriptome analysis to determine the different pathways of the physiology of NK cells that could be altered in polycythemia vera. NK cells were defined according to the classical phenotype CD3-CD16 + and CD56 + and isolated from healthy donors (controls) and patients with polycythemia vera (after obtaining their written consent). **Results.** Our preliminary data (Sanchez et al. Manuscript in preparation) showed a deficit of cytotoxic functions of NK cells despite a normal or even increased, there is no deficit of activating molecules and cytotoxic potential (perforin / granzyme) intact. This suggests that the functional deficit of NK could be related to one or abnormalities of signaling pathways. This led us to conduct an on-chip Agilent transcriptome, by comparing NK purified from patients with polycythemia vera compared with healthy subjects. Hierarchical clustering revealed a hundred differentially expressed genes and completely separated patients with disease from healthy controls. Several genes upregulated in patients emerge from the list as OSBP2, NELL2, while others are under expressed as IRF8, TNFRSF1B and SP3. **Conclusions.** The first results obtained show a list of genes differentially expressed between patients and controls. We continue the analysis of the different clusters and we construct a dynamic in silico model system for regulating cytotoxic functions of NK cells to explain the origin of their dysfunction in polycythemia vera.

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T REGULATORY CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background. Primary myelofibrosis (PMF) is a clonal, neoplastic disorder of the hematopoietic stem cells, characterized by bone marrow fibrosis, anemia, increased number of circulating CD34+ cells and splenomegaly. Besides astenia, fever, pruritus and weight loss, a subset of patients display immune-related abnormalities that suggest an (auto)immune pathogenesis, such as antibodies to RBCs, ANA, AMA, RF, low levels of complement, and lupus-like anticoagulant. In addition, it has been reported a significant association between personal history of a broad span of autoimmune diseases and subsequent risk of myeloproliferative neoplasm (Kristinsson SY Haematologica 2010; 95:1216-1220). T regulatory cells (Tregs) are an immunosuppressive T cell subset that prevents autoimmunity, regulate T-cell homeostasis, and modulate immune responses against a variety of pathogens. The involvement of Tregs in blood diseases have been studied and there is accumulating evidence that these cells play a role in hematopoietic activity (Moon HW Korean J Lab Med 2011;31:231-237). **Aims.** to investigate the number of circulating Tregs, and to correlate them with main parameters of the disease in patients with PMF, disease controls with polycythemia vera (PV) or essential thrombocythemia (ET), and in healthy controls (CTRLs). **Methods.** We tested 44 patients with PMF (pre-fibrotic type, n=10, fibrotic type, n=28, post PV/ET MF,n=6) 5 with PV/ET, and 11 healthy subjects (CTRLs). All the subjects included in the study signed the informed consent. According with data from the literature, Tregs were evaluated as CD4+CD25+CD127low/neg cells by cytofluorimetric analysis of peripheral blood mononuclear cells (PBMNCs). Fivex10^6 cells were stained by PerCPCy5. 5-anti-CD4, Alexa488- anti-CD25, PE-anti-CD127 monoclonal antibodies (eBioscience). The evaluation of FoxP3, the molecule that definitely identify the regulatory function of a T cell, was performed by intracellular staining using the commercial fixation -permeabilization kit (eBioscience); the 70-90% of the CD4+CD25+CD127 low/neg cells were FoxP3+ (Koreth J NEJM 2011 365:2055-2066). Results, expressed as percentage of CD4+ cells, are shown as median (range). **Results.** The percentage of CD4+CD25+CD127low/neg Tregs was not significantly different among patients with PMF (1. 3%, 0. 1-6. 7), PV/ET (0. 7%, range 0. 1-4. 9) and CTRLs (1. 8%, range 0. 8-5. 3). No significant correlation was found between the percentage of Tregs and disease parameters such as disease duration, severity score, WBCs, PLTs, and IWG-prognostic score. When patients with PMF were divided according to the Hb levels, those with less than 10g/dl (n=8) had a percentage of circulating CD4+CD25+CD127low/neg Tregs (0. 8%, 0. 1-1. 7) significantly lower (p= 0. 02) than those with Hb levels equal or higher than 10g/dl (n=36) (1. 5%, 0. 1-6. 7), and those of CTRLs (1. 8%, range 0. 8-5. 3) (p= 0. 02). **Conclusions.** These preliminary data indicate a correlation between a low percentage of CD4+CD25+CD127low/neg Tregs and anemia in patients with PMF. It is well known that anemia in patients with PMF is a result of ineffective erythropoiesis, intra-vascular hemolysis and bleeding. However, data regarding an improvement in anemia following immuno-suppressive therapies, suggest a possible role of autoimmunity as a cause of anemia in patients with PMF.

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EFFECTOR / MEMORY CD4 POSITIVE CELLS PREVAIL IN THE LYMPHOCYTE POPULATION OF ESSENTIAL THROMBOCYTHEMIA (ET) PATIENTS

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Patients with ET were investigated for selective trapping/expansion of T lymphocytes. For that, subpopulations of T lymphocytes were measured in blood and in marrow specimens. The following T cell subpopulations were determined: CD45RO-CCR7+, CD45RO+CCR7+, CD45RO+CCR7- for naive, central memory, effector memory and TEMRA cells respectively. The whole population of marrow and blood cells were labeled for the purpose of this study with the use CD4, CD25, CD45RO and CCR7 MoAb (Becton Dickinson, San Jose, CA, USA) followed by detection and analysis with the use of flow cytometry (FACS Calibur, Becton Dickinson) equipped with PCLysis program. Nineteen patients (F/M: 14/5, age 28-78 - median 60 yrs) with ET diagnosed according to the WHO criteria were studied. The platelets in blood ranged from 503 to 884 x10^3/ul (median 676 x 10^3), NAP score from 44 to 202 (median 104). 15 patients were positive for JAK 2 V617F mutation (13 heterozygotes, 2 homozygotes). Marrow cellularity was from 5 to 126 x10^3/ul (median 38 x 10^3/ul). Lymphocytes in the marrow were in proportions from 4,0% to 21% (median7,5 %, 3386/ul). Lymphocytes in blood ranged from 1300/ul to 4300/ul (median 2050/ul). Cytological evaluation of marrow aspirates revealed normal differentiation of erythroid and myeloid lineages, in contrast increased numbers of megakaryocytes, often enlarged, hyperlobulated and in clusters were found. In trephine biopsy specimens megakaryocytes were enlarged but other cell lineages looked rather normal and there was no fibrosis. Results.1. Proportions and numbers of naive T cells were similar in blood and in marrow2. CD45RO+CCR7- prevailed in marrow as compared to blood (141,6 - 3241,3 /ul, median 1281/ul vs 68,7 - 2437,5 /ul, median 631,2/ul, p=0,04 Wilcoxon Test for pairs). 3. there was a significant increase of CD4+CD25+ cells in the marrow as compared to blood (4,5-169,7/ul, median 36,2/ul vs 1,2-47/ul, median 9,1/ul; p=0,02 Wilcoxon Test for pairs) . Trephine biopsy investigation documented a high frequency of FOXP3 positive cells. In conclusion a selective increase of effector/memory CD4 lymphocytes (CD45RO+CCR7-) and their counterpart population of T regulatory (CD4+CD25+) cells suggests the presence of immune response in the marrow of ET cases

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ERG EXPRESSION IN A COHORT OF CHILDREN WITH MYELOPROLIFERATIVE DISEASE

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Background. ERG gene is involved in the pathogenesis of many cancers. ERG is necessary for haemopoiesis, angiogenesis, inflammation regulation

and osteogenesis. Recently, literature has shown ERG role in the regulation of hematopoietic stem cells and embryonic and adult megakaryocyte maturation. In particular, the overexpression of ERG induces an increase in STAT3 phosphorylation and increases intracellular levels of JAK2 and STAT5. In hematology ERG expression has been analysed only in cases of pediatric and adult acute leukemia. **Aims.** the study evaluates the role of ERG in the pathogenesis of myeloid proliferation in children followed for Essential thrombocytemia (ET), primary polyglobulia (PG) and Chronic Myeloid Leukemia (CML). ET and PG, quite rare in children, are myeloproliferative neoplasm (CMPN), but since they are not characterized by a specific marker of clonality, are difficult to classify. **Materials and Methods.** in 58 children (15 ET, 19 PG and 24 CML) aged less then 18 years, ERG expression was evaluated by Real Time PCR and compared to the expression in 14 healthy controls. ERG overexpression was detected when RQ ratio was >1. Within PG children, 2 children, brother and sister, had a congenital form; 1 child had father and grandfather with the same problem; 4 children had Down Syndrome (DS); 1 child had VHL mutation (Chuvash PG); 1 high oxygen affinity hemaoglobin. 4/15 ET children had JAK2V617F mutation and 1 had MPL W515S mutation**.** CML patients (all bcr/abl p210 positive)were analyzed both at diagnosis and in remission. **Results.** four out of the 19 children (21%) with PG had ERG overexpression, with no significant correlation with the PG characteristics. ERG overexpression was found in 7 ET (46%) and 3 of them (43%) carried JAK2V617F mutation. In contrast, within those with normal ERG expression only 1 was JAK2V617F (12. 5%). A slight correlation was found (albeit not statistically confirmed) with ERG overexpression and WBC count > 10 x 10^9/l. ERG elevated overexpression was found in the peripheral blood cells (PB) of all patients with CML, comparing to healthy children; in the bone marrow (BM) cells, ERG was expressed at the same level than HS and interestingly, at the same level than PB. CD34+ cells sorting showed that ERG expression was confined to this subset. CML analyzed during treatment with tyrosin kynase inhibitors showed a complete return to normal levels. **Conclusions.** ERG is heterogeneously expressed in these children; in PG, ERG expression is not helpful in directing pathogenetic suggestions. Clonal myloproliferation in CML is associated with CD34+ cells ERG overexpression and this is suppressed by TK inhibitors. In ET, correlation with JAK2V617F is suggested. Extension of this study is required to understand the role of ERG in myeloid proliferation, both clonal and non clonal**.**

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THE INCIDENCE OF JAK2 V617F MUTATION IN RADIATION-ASSOCIATED BCR/ABL-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS FOLLOWING THE CHERNOBYL ACCIDENT

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Background. A recurrent specific JAK2 V617F mutation has been reported in bcr/abl-negative chronic myeloproliferative disorders (cMPDs), including polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF). The implication of this mutation in the pathogenesis of cMPDs is currently confirmed. Our study is the first to be interested in the status of the JAK2 V617F mutation in patients with cMPDs developed after radiation exposure. **Aims.** The aim of the study was to analyze whether radiation-associated cMPDs developed in clean-up workers at the Chernobyl NPP and inhabitants of Ukrainian territories with high contamination from radionuclide fallout differed from spontaneous cMPDs in terms of JAK2 V617F incidence. **Methods.** The cohort of patients in the study consisted of 118 unselected adult cMPDs patients, initially diagnosed between 2001 and 2012. Of these patients, 37 had experienced radiation exposure due to the Chernobyl accident (19 clean-up workers and 18 inhabitants of Ukrainian territories with high contamination from radionuclide fallout) and composed the group of radiation-associated cMPDs, and 70 developed spontaneous cMPD and served as controls. After informed consenting, mutation screening for JAK2 V617F in patients with PV, ET and IMF was performed by allele-specific polymerase chain reaction assay. **Results.** JAK2 V617F mutation was detected in 89 of the 118 patients with cMPDs. In particular, 95. 2, 50 and 55,3% of PV, ET and IMF cases, respectively, showed the mutation. The frequency of JAK2V617F mutation was similar among radiation-associated and spontaneous PV (18 of 19 vs. 41/43, *p*=0,92) and ET (1/2 vs. 8/16, *p*=1,0) cases, but it was lower in radiation-associated IMF than that in spontaneous IMF with the difference on the border of statistical significance (6/16 vs. 15/22 patients, *p*=0,06). **Summary and Conclusions.** We assess the status of the JAK2 V617F mutation in patients with radiation-associated cMPDs following the Chernobyl accident. Our data seem to confirm that the JAK2 V617F mutation incidence is similar in radiation-associated and spontaneous PV and ET patients. However, our findings are suggestive that JAK2 V617F mutation may by less common in radiation-associated compared to spontaneous IMF. The further studies are needed to provide insights into understanding of the molecular basis of radiation-associated cMPDs.

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FREQUENCY AND CLINICAL CORRELATES OF JAK2 46/1 HAPLOTYPE IN COMPARISON WITH JAK2V617F VARIANT IN ESSENTIAL THROMBOCYTHEMIA: SINGLE CENTER EXPERIENCE

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It is predicted that the inherited genetic background in the individual patients with myeloproliferative neoplasm (MPN) influences the disease susceptibility and the phenotype expression of the MPN. Several groups discovered that the germline JAK2 haplotype, tagged by the “C” allele of single nucleotide polymorphism (SNP) rs12343867 (C/T) is associated with the JAK2V617F positive MPN. Also, some recent studies showed equal distribution of this SNP among JAK2V617F negative MPN and questioned the role of this hyplotype in MPN patients. In order to extend further those observations we conduct a retrospective study. First, we assess the frequency of JAK2 46/1 haplotype in Essential thrombocyhemia (ET) patients in comparison with population controls. As second we evaluate the association of 46/1 with the JAK2V617F mutational status and the clinical characteristics in the series of patients with ET that were diagnosed and treated at the University Clinic of Hematology-Skopje, Republic of Macedonia. Ninthly five consecutive patients diagnosed with ET according to proposed criteria for diagnosis in 2008 by the World Health Organization were included in our study. The 46/1 tag SNP rs12343867 (C/T) was genotyped on a MxPro 3005P real-time PCR system (Stratagene, La Jolla, CA,USA) using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. The incidence of 46/1-linked C allele was significantly higher in ET (genotype: CC 13%, CT 60%, TT 27%; C-allele frequency: 43,7) than in population control (C-allele frequency 29%), P<0,01. Genotype distribution were similar among JAK2V617F positive/ JAK2V617F negative patients (genotype: CC 7/14%, CT 22/29%, TT 67/57%; C-allele frequency: 41/43%; P=0,76). The clinical characteristics of 46/1 positive and negative ET were comparable regarding all tested parameters such as blood counts, NAP score, rate of thrombotic and hemorrhagic complications, disease transformation and survival. Our results confirmed latest observations that JAK2 46/1 haplotype is susceptibility factor for developing ET independent of JAK2V617F mutational status and does not further affect the clinical course and prognosis of the disease.

1602

KL-VS FUNCTIONAL POLYMORPHISM OF THE KLOTHO GENE IS NOT ASSOCIATED WITH THE MYELOPROLIFERATIVE NEOPLASMS PHENOTYPES, BUT IT MIGHT MODULATE THE ACQUISITION RISK OF SOMATIC MUTATION JAK2 V617F

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Background. KL-VS functional polymorphism of the *KLOTHO* gene has been associated with longevity, and with a large spectrum of chronic degenerative diseases, or more recently with diverse cancers. Polycythemia vera (PV) and essential thrombocythemia (ET) are representative non-BCR-ABL myeloproliferative neoplasms (MPN), frequently positive for the somatic mutation *JAK2* V617F. **Aims.** To evaluate the relationship between the KL-VS polymorphism of the *KLOTHO* gene, and the occurrence of the MPN phenotype, and further between this polymorphism and the *JAK2* V617F somatic mutation. **Methods.** One hundred sixty-six patients with MPN (75 with PV and 91 with ET) with a known *JAK2* V617F status, and 95 controls were included in the study. The KL-VS polymorphism was genotyped in all patients and controls by a PCR-RFLP assay. **Results.** Thirty-three patients (19. 9%) had at least one KL-VS allele. There were 29 heterozygotes (17. 5%) and 4 homozygotes (2. 4%). Among the controls, there were 20 individuals harbouring at least a KL-VS allele (21. 1%), of which 19 heterozygotes (20%) and one homozygote (1. 1%). There was no significant difference regarding the distribution of the KL-VS polymorphism in patients compared to controls (OR = 0. 9; 95% CI = 0. 5 - 1. 5; p-value = 0. 74). In the whole cohort of patients, there were 118 patients (71. 1%) positive for

JAK2 V617F mutation. After stratifying for the *JAK2* V617F status, we found that the KL-VS polymorphism was enriched in V617F-positive compared to V617F-negative patients (OR = 2. 3; 95% CI = 0. 9 - 5. 5; p-value = 0. 05). **Summary and Conclusions.** Our preliminary findings suggest no significant relationship between the functional polymorphism KL-VS from the *KLOTHO* gene and the occurrence of MPN phenotypes (PV and ET). However, we found this polymorphism more frequently among patients positive for the *JAK2* V617F mutation, the most common molecular marker in PV and ET. Although the mechanism is unclear, this finding suggest a possible contribution of the KL-VS polymorphism to the risk of acquiring the V617F mutation.

1603

THE 'C' ALLELE OF JAK2 RS12343867 DESIGNATE THE GENETIC BASIS OF MYELOPROLIFERATIVE NEOPLASM IN THE MACEDONIAN POPULATION

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Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are myeloproliferative neoplasms (MPNs) characterized in most cases by a unique somatic mutation *JAK2* V617F. Recent studies suggested that JAK2V617F positive MPNs are acquired preferentially on a specific constitutional germline JAK2 46/1 haplotype(46/1)which is tagged by the "C"allele of single nucleotide polymorphism (SNP)rs12343867 (C/T). Moreover, some studies investigated the potential role of the JAK2 46/1 haplotype at the MPN phenotype in context of the clinical presentation and the complication of the diseases. Our study was extend in order to examined the impact of the C allele of JAK2 rs12343867 (C/T) on MPNs in a Macedonian population and to evaluate the association of 46/1 with the JAK2V617F mutational status and the clinical characteristics in our MPNs patients. The study group consisted of 212 adult (>15 years) patients with MPNs that were diagnosed and followed at the University Clinic of Hematology- Skopje. According to the 2008 World Health Organization criteria 79patients were diagnosed as PV, 95 as ET, 10 as PMF and 28 were classified as atypical MPNs (aMPNs). The 46/1 tag SNP rs12343867 (C/T) was genotyped using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The JAK2 V617F mutation was analyzed by fluorescent allele-specific PCR followed by CE on ABI 310 Genetic analyzer. Associations with risk of MPN were estimated by odds ratios and their 95% confidence intervals using logistic regression. The incidence of 46/1-linked C allele was significantly higher in all MPN entities [PV (0. 538), ET (0. 437), PMF (0,464), and in aMPNs (0. 55)]in comparison with healthy controls (0. 290); (P<0,01 for all comparisons). The frequency of the JAK2V617F mutation ranged from 89%in PV, 67% in ET, 60% in PMF to 46,4% in the aMPNs. The frequency of the JAK246/1 C allele was significantly higher in the JAK2V617F positive patients with PV, PMF and aMPN; (p<0,01 for all comparisons) except in ET patients, in which genotype distributions were similar among JAK2V617F positive and JAK2V617F negative patients (genotype: CC 7/14%, CT 22/29%, TT 67/57%; C-allele frequency 41/43%; p=0,76)Correlations of the clinical features at diagnosis and long-term prognosis between the two JAK2 46/1 different MPNs groups revealed only that in ET patients risk for thrombosis (artherial and venous) was significantly associated with *JAK2* V617F mutation(38,5%;(P<0. 005), rather than with the 46/1. Our results confirmed latest observations that JAK2 46/1 haplotype contributes significantly to the occurrence of of *JAK2* V617F-positive and *JAK2* V617F-negative MPNs in the Macedonian population and it designate the genetic basis for predisposition to MPNs. They also showed that the JAK2 46/1 haplotype does not affect the clinical course and prognosis of the different MPNs.

1604

ATYPICAL MYELOPROLIFERATIVE DISORDER WITH BASOPHILIA, TRISOMY 4 AND HIGH TRYPTASE LEVELS

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Background. Hematologic disorders with basophilia are so uncommon that there is not an specific entity for them, with exception of AML with recurrent t(6;9). We present the unique case of an asymptomatic female with blood basophilia, marrow basophilia and eosinophilia, myelofibrosis and amplification of PDGFRA due to trisomy 4. **Description.** 52 yr. -old female. In August/11 she underwent partial hepatectomy for a single metastasis, which primary was a cervical cystic adenoid carcinoma treated in 2008 with surgery and local radiation. In Oct. -11, significant basophilia was found in an hemogram. At this moment, she only described mild weight loss; she denied, fever, night sweats, recent skin lesions, itching, asthma, urticaria or episodes of angioedema/anaphylaxis. Physical exam: no hepatosplenomegaly / lympadenopathies. Lab. tests:- Hemogram: 4. 1· 10^9 leuc. /L; Hgb., 11'6 gr /dL; fL; 208· 10^9 plat. /L. Blood smear: 23% neutr.; 50% basophils, a minority of which with irregular distribution of granules; no mast cells. - Biochemistry: ALT, AST, LDH, creatinine, proteins within normal range. ESR, 15. Vit. B_{12} > 1500 pg/mL. Ferritin, 161 ng/mL. Reticulocytes 1. 89%. - Tryptase, 166 mg/dL (normal<12). Histamine, 0'89 mg/dL (N). Bone marrow exam. Aspirate: significant dysplasia in megakayocytic and erythroid lines; 27% basophils; 1% blasts. Biopsy: intertrabecular spaces fully occupied by a marked myeloid hyperplasia; 30-40% eosinophils in all maturative stages; % of blasts / CD34+ cells was normal; sparse mast cells not forming infiltrates; reticulinic myelofibrosis with focal areas of collagen. FISH: 80% cells with amplification of PDGFRA(4q12); no amplification of ETV6 (12p13); absence of t(9;22) and abnormalities in 5q31, 7q31, 8, 20q12. Cytogenetics: 48, XX, +4, +19 [20]. Molecular biology: absence of V617F· Jak2 and bcr/abl; absence of mutations of c-kit in basophils selected by sorting. HUMARA test: clonality. Flow cytometry: 1. 3% CD34+; absence of abnormal mast cells; 34% of aberrant basophils in a intermediate stage of maturation, CD45+, CD117+, CD25+, IgE++, CD38++, DR-, CD11b-, CD13+, CD16-, CD10-, CD34-, CD35+, CD64-, CD14-, Tdt-, CD56-, CD19-, CD3-, MPO-, CD22+, 7. 1 -, CD15-, CD123+++, CD203c-, CD33++. CT exam (after surgery): no splenomegaly; mild volume of intraperitoneal fluid; no pleural effusion. **Summary and Conclusions**. To sum up, this patient had a clonal proliferation of basophils, bone marrow eosinophilia and myelofibrosis, very high levels of serum tryptase (x12) and trisomy 4 without identified traslocations involving PDGFRA. These data allowed us to rule out mast cell disease, chronic myeloid leukemia and t(6;9) AML. However the adscription within WHO entities remains uncertain: "unclassifiable myeloproliferative or myelodysplastic / myeloproliferative neoplasm", "myeloid neoplasm with eosinophilia and abnormalities of PDGFRA", the last being the most appealing, even though the "abnormality"is a trisomy instead of a traslocation. This should not be an excluding criterion, since there is at least one analogous case of complex karyotpe involving chr. 4 (Score J. *Leukemia 2006;80,827*). The very high levels of tryptase, in the rank of mast cell disease, were the other remarkable fact (although expected), in the absence of allergic manifestations. Considering the lack of clinical expression, the only recommended therapy has been anti-histamine drugs (ebastine and ranitidine).

1605

PROGNOSTIC SIGNIFICANCE OF FLT3 AND NPM1 MUTATIONS IN MYELOID MALIGNANCIES

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Background. The fms-like tyrosine kinase 3 (FLT3) protein is a tyrosine kinase receptor wich gene is mutated in about one third of acute myeloid leukemia (AML) patients, being the *FLT3* internal tandem duplications (ITD´s) mutations associated with an adverse prognosis in this disease. In the long arm of chromosome 5 (5q35) is localized the gene that encodes a nucleolar phosphoprotein, Nucleophosmin (NPM1), which mutation in exon 12 is found in about a half of AML patients, giving a better prognosis. The mutational status role of these genes in Myeloproliferative Neoplasms (MPN) and Myelodysplastic Syndrome (MDS), is not well defined, namely their´s role as a potential marker of progression to AML. **Aims.** In this study was analyzed the frequency of the *NPM1* and *FLT3* genes in patients with AML, MPN and MDS, cytogenetically characterized, and analyzed their correlation with clinical parameters, disease progression, treatment response and prognosis/survival. **Methods.** To achieve our objectives, the *FLT3* and *NPM1*genes mutations was performed on genomic DNA extracted from peripheral blood (PB) or bone marrow (MO) samples of 76 patients with myeloid malignancies, including 52 with AML diagnosis, 19 with MNP´s and 5 with MDS, according to FAB (French-American-British) criteria and World Health Organization (WHO) (2008). Detection of *FLT3* gene mutations, in particular the Internal Tandem Duplications (ITD) and the D835 mutation was achieved after amplification by PCR (Polymerase Chain Reaction) and by RFLP (*Restriction Length Polymorfisms*) analysis, respectively, while the search for mutations of *NPM1* gene (exon 12) was carried out by gene sequencing. **Results.** The presence of *FLT3*-ITD and exon 12 of *NPM1* genes mutations were only identified in AML patients, while none of the MPN and MDS patients carried out this kind of mutation. However, in two MPN patients we found the NPM1 and in one D835 FLT3 mutation. The incidence of FLT3-ITD

and NPM1 exon-12 mutations in AML patients was 28 and 9,6%, respectively. These patients had higher white blood cell count and percentage of blasts. Our results show that patients positive for both mutations (*ITD +/NPM1 +*), were those in which the rate of complete remission (CR) was lower (34%), compared with that seen in patients without mutations (*ITD-/NPM1-*), or with those in witch only one mutation was identified (*ITD-/NPM1+* or *ITD+ / NPM1)*. The highest rate of CR was achieved in patients *ITD-/NPM1 +* (100%). The overall survival (OS) was much higher in patients without the *ITD* gene mutation (*FLT3/ITD* negative) as well as in patients with *NPM1* gene mutation (*NPM1* positive). **Conclusions.** These results suggests that the ITD mutation in the *FLT3* gene confers a worse prognosis for AML patients enrolled in the study, while *NPM1* gene mutations confers a favorable prognosis for these patients. Since the frequency of these genes mutations are more common in AML patients and confer distinct prognosis, early detection contribute to prognosis assessment and to individualized therapeutic approach directed at molecular targets. The detection of these mutations in patients with MPN and MDS may also have prognostic relevance, but larger studies are needed.

1606

JAK2 MUTATIONS IN CHRONIC MYELOPROLIFERATIVE NEOPLASM; TOWARDS THE APPLICATION OF PERSONALIZED TREATMENTS FOR SAUDI PATIENTS

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Background. The chronic myeloproliferative neoplasms (CMPN) are a group of clonal hematopoietic stem cell disorders in which large numbers of red blood cells, white blood cells, or platelets grow and spread excess in the bone marrow and the peripheral blood. Cytogenetic analysis of the t (9:22) and molecular detection of BCR-ABL is the main diagnostic criteria in Philadelphia positive CMPN (CML). The identification of non-receptor tyrosine kinase JAK2 mutations (exon 14 JAK2 V617F and exon 12) have significantly contributed to our understanding of the molecular mechanisms in the pathogenesis of Philadelphia negative CMPN such as polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (MF) patients. According to the revised WHO classification, JAK2 mutation is considered as a major diagnostic and clonal marker in Philadelphia negative CMPN which will play a major role in designing personalized treatments for the disease. **Aims.** To determine the frequency of JAK2 mutation in myeloproliferative neoplasms in western region of Saudi Arabia. **Methods.** JAK2 V617F mutation frequency is unknown in Saudi Arabia. Therefore, investigation of the JAK2 V617F mutation was carried out in DNA samples from 78 peripheral blood specimens corresponding to patients with polycythemia vera (PV) (n = 11), Chronic myeloid leukemia (CML) (n=45), essential thrombocythemia (ET) (n = 10), Primary myelofibrosis (PMF) (n = 12). We used polymerase chain reaction and direct DNA sequencing to detect the JAK2 mutation. **Results.** Overall, the incidence of the JAK2 V617F mutation was 91% in PV, 40% in ET, and 25% in PMF. This approach proved to be reliable and more sensitive in detecting the mutation. Two essential findings arose from our study. First, this technique could be carried out with DNA samples, even partially degraded, from routinely processed BM or peripheral blood specimens. Second, after correlation with morphological features, it turned out that the characteristics of the megakaryocytes were more specific than the mutational status of JAK2 in characterizing ET and PMF. **Conclusions.** Concerning PV, as expected, the incidence of the JAK2 mutation was higher, but the morphological criteria were misleading in some cases, strongly suggesting that the combination of both morphology and molecular data would enable the characterization of virtually all cases. JAK2 V617F mutation frequency along with accurate morphological characterization is very reliable tool in diagnosing and classifying CMPN in Saudi patients.

1607

PATIENTS' COMORBIDITIES AND OVERALL SURVIVAL IN PRIMARY MYELOFIBROSIS (PMF)

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Background. Cancer patients often experience comorbidities that may affect their therapeutic options, outcome of therapy, and their prognosis. However, none of the prognostic scoring systems for PMF account for an impact, if any, of patients' comorbidities on their prognosis. **Aims.** The aim of this study was to determine the effect of comorbidities on the survival of patients (pts) with PMF. **Methods.** We reviewed the medical records of 349 consecutive PMF pts who presented to our institution from 2000 to 2008. The Adult Comorbidity Evaluation-27 (ACE-27) form, a validated 27-item comorbidity index for cancer pts, was used to assess the severity of comorbid conditions in these patients at the time of presentation. Demographic characteristics including age, performance status (PS), complete blood profile, karyotype, International Prognostic Scoring System (IPSS), stem cell transplant (SCT) and outcomes (leukemic transformation and survival) was also collected. Kaplan-Meier method and log-rank test were used to assess survival. Multivariate analysis (MVA) was performed using the Cox Proportional Hazards Model. **Results.** Of the 349 pts included in this study, 222 (64%) were male and 311 (89%) were white; median age at presentation was 62 years (20-82); median duration of follow-up was 37 months (range 0 - 133). The ACE-27 comorbidity scores were as follows: none, 125 pts (36%); mild, 128 (37%); moderate, 63 (18%); and severe, 33 (9%). Hypertension was the most common comorbidity (31%) followed by diabetes mellitus (14%). A total of 193 patients died, 41 (12%) suffered leukemic transformation and 27 (8%) patients underwent SCT. Overall median survival using the Kaplan-Meier method was 39 months. Median survival by ACE-27 scores was: 52, 38, 36 and 28 months for none, mild, moderate and severe comorbidity scores, respectively (p=0. 028). In addition to comorbidities, age >65 years, male gender, increased PS (by Zubrod Scale), white cell count >25K, platelets <100K, hemoglobin <10 g/dl, transfusion need, albumin <3. 5 g/dl, unfavorable karyotype, prior treatment, and increased IPSS risk reached statistical significance for survival (p<0. 05). Constitutional symptoms and peripheral blasts did not achieve statistical significance. In the MVA, advanced age (>65 years), PS of >=2, platelet <100K, transfusion need and IPSS (high risk category) continued to maintain prognostic significance. Comorbidities were not identified as an independent predictor of survival. However, significant interaction was noted between age and comorbidities (p<0. 01). Hence additional analysis excluding age was performed that identified comorbidities as an independent predictor of survival (p=0. 03). When stratified by age, patients <=65 years with comorbidities were noted to have a significantly lower survival (p=0. 02). A significant trend was also observed between the severity of ACE-27 comorbidity scores and survival in patients <=65 years (p=0. 001). In older patients (>65 years), comorbidities failed to predict survival. **Conclusions.** We observed significant effect of comorbidities on survival in patients with PMF <=65 years of age. Our study suggests that interventions aimed at improving comorbidities in younger patients with PMF may improve their overall outcome.

1608

A RETROSPECTIVE ASSESSMENT OF THE CLINICAL FEATURES AND SYMPTOMS OF 180 PATIENTS WITH MYELOFIBROSIS IN THE UNITED STATES

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Background. Myelofibrosis (MF) is a life-threatening myeloproliferative neoplasm characterized by debilitating symptoms (eg, fatigue, night sweats, pruritus, abdominal pain, and early satiety). Some patients also present with an enlarged spleen (splenomegaly), further adding to their disease burden. **Aims.** To characterize the clinical features and symptom profile of MF in patients with and without splenomegaly. **Methods.** This was a retrospective chart review conducted in April and May of 2011. Patients diagnosed with MF (primary MF, post-polycythemia vera MF, or post-essential thrombocythemia MF) between January 1, 2005 and March 31, 2010 were included in the analysis. The study was designed so that approximately half of the patients did not have splenomegaly (defined as any spleen enlargement palpable below the left costal margin). Patients were required to have at least 12 months of chart data following diagnosis of MF, and patients with splenomegaly were required to have an additional 12 months of chart data following first detection of splenomegaly. MF-related symptoms, including items from the Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF), and treatments received were collated. Data collection occurred before the FDA approval of ruxolitinib for the treatment of intermediate or high-risk MF. **Results.** Charts for 216 patients were contributed by 57 physicians in the United States (80% hematologists/oncologists; 66% community practice). Data for 180 patients (102 with and 78 without splenomegaly) were eligible for analysis. Patients had a median age of 66 years, and 82% presented with intermediate-2 or high-risk MF by International Prognostic Scoring System criteria. All patients had significant comorbidities at the time of their diagnosis, most commonly hypertension, diabetes, dementia, and cardiovascular disorders. Patients with and without splenomegaly also reported a high frequency of MF-related symptoms at baseline. The occurrence of constitutional symptoms was similar in patients

with and without splenomegaly (weight loss 78% vs. 69%, night sweats 65% vs. 50%, and fever 54 vs. 53%, respectively); however, abdominal symptoms and early satiety were significantly (p<0. 05) more frequent in patients with splenomegaly (range 67% to 85%) vs. those without splenomegaly (range 37% to 50%). Most patients reported fatigue (93% and 87% with and without splenomegaly, respectively). Approximately half of the patients received treatment for MF during the time frame of the chart review, the most common being hydroxyurea and growth factors (including erythropoietin-stimulating agents). **Summary and Conclusions.** This large retrospective study of patients with MF demonstrated a high prevalence of comorbid conditions and debilitating MF-related symptoms, which occur regardless of the presence or absence of splenomegaly. These findings support current efforts to improve the treatment of MF by targeting both disease symptoms and spleen size. **Supported.** *Supported by Incyte Corporation. Dr. Mughal is a consultant for Incyte Corporation.*

1609

THE ROLE OF JAK2-V617F MUTATION IN LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS

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Background. AND OBJECTIVES:JAK2 mutation is recurrent in myeloproliferative neoplasms (MPNs) and it is almost invariably associated with PV, but it occurs in the majority of patients with ET or PMF. The role in the pathogenesis of the various BCR-ABL1-negative myeloproliferative neoplasms (MPNs) remains unclear and its significance in leukemic transformation is a matter of even greater controversy. **Aims.** The aim of this study was to evaluate both the JAK2(V617F) mutational status of the rare cases in which blast crisis occurred in our institution and the response after intensive treatment. **Materials and Methods.** Between 2000 and 2011, 194 patients received diagnoses of BCR-ABL1-negative MPNs in our center (68 with PV, 50 with ET, and 59 PMF cases, as well as 13 MPN cases not otherwise classifiable). Of these patients, 21 developed leukemic transformation (5 with MF post PV, 3 with MF post TE,13 with PMF). The genotyping of the JAK2(V617F) mutation was performed by the amplification-refractory mutation system. **Results.** All the patients were tested for JAK2(V617F) in the chronic phase of their disease, and 18 of these patients were positive for JAK2(V617F). These 18 patients, 2 with PV, 4 with ET and the rest with PMF, also harbored JAK2(V617F) in the heterozygous state during blast crisis and even after intensive treatment in one of these patients. The other 3 cases that evolved to blast crisis did not harbor the JAK2(V617F) mutation before and after transformation. 20 patients died despite conventional or supportive treatment and 1 patient is still under treatment. **Conclusions.** The transformation of MPNs into acute leukemia is a rare phenomenon. In our study, all JAK2(V617F)-positive patients remained positive for this mutation after leukemic transformation, although in the heterozygous state, suggesting that JAK2(V617F) is not essential for transformation in these cases. The fact that all JAK2(V617F)-negative cases remained negative after blast crisis reinforces the theory that other molecular event(s) may play a role in the clonal heterogeneity of MPNs. Regarding the treatment, conventinal or supportive treatment do not seem to be a solution because of the poor outcome of acute myeloid leukemia secondary to MPN, so patients should be included in clinical trials of the novel JAK2 inhibitors.

1610

ESSENTIAL THROMBOCYTHAEMIA (ET): DISEASE SUBGROUPS AND PROGNOSTIC FACTORS IN 181 PATIENTS FROM A SINGLE CENTER.

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Background. Essential thrombocythaemia (ET) is the most frequent myeloproliferative disorder characterized by a long natural history. The identification of JAK2V617F mutation facilitates the diagnosis and probably defines two distinct disease subgroups. **Aims.** Aim of the present study is to report the clinical and laboratory findings, the outcome, potential prognostic factors as well as the clinical importance of JAK2V617F mutation in patients with ET. **Methods.** 181 patients diagnosed with ET in a single center were retrospectively studied from 1998 to 2011. **Results.** The median age of our patients was 64 years (16-91), 72 were males and 109 females. At diagnosis 75% were asymptomatic, 17% presented either a thrombotic or a bleeding episode and 11% had splenomegaly. The JAK2V617F mutation was detected in 64/95 patients (67%). The median values of Ht, Hb, WBC and PLT were 42. 6% (30. 8-54. 7), 13. 9g/dL (9. 5-17. 3), 8. 48 x 10^9/L (4. 44-27. 4) and 781 x 10^9/L (468-2288) respectively. LDH levels were elevated in 25%, while the median serum erythropoietin level was 10. 2IU/mL. Bone marrow biopsy revealed the presence of typical megakaryocytes in clusters in 75%, while reticulin fibrosis was found in 29% with 8 cases showing grade 2 fibrosis. An abnormal karyotype was evident in 9%. The comparison between JAK2V617F(+) and JAK2V617F(-) patients showed that JAK2V617F(+) patients presented more frequently a major event at diagnosis (p=0. 05), had statistically significantly higher values of Ht (median: 44. 9% vs 39. 9% p<0. 001), Hb (median: 14. 5g/dL vs 13. 1g/dL p<0. 001), lower platelet counts (median: 698x10^9/L vs 892x10^9/L and lower MCV (median: 84. 5fl vs 88. 2fl). Antiplatelet treatment was administrated in 78%. Cytotoxic therapy was given in 80%, while the remaining patients were put under observation. The median interval from diagnosis to the initiation of cytotoxic therapy was 0. 8 months (0-115). First line treatment was hydroxyurea in 86%, anagrelide in 12% and interferon-a in 1%. Complete and partial response was achieved in 68% and 26% of the patients respectively, at a median time of 1. 64 months. Change of treatment was required in 11%. At a median follow up of 40 months (1. 1 - 207), 94% of the patients are alive with a 5- and 10-year overall survival (OS) of 96% and 87% respectively. Events were observed in 13 patients: 6 experienced a thrombotic episode, 5 progressed to myelofibrosis and 2 developed a second malignancy. Prognostic factor analysis revealed male gender (p=0. 001) and WBC counts ≥10X10^9/L (p=0. 001) as adverse factors for OS. Bone marrow fibrosis and the presence of an event at diagnosis were of marginal significance (p=0. 09). Furthermore, Hb<12g/dl and PLT<600X10^9/L at diagnosis correlated with a statistically significantly increased risk for the development of a thrombotic event *(p=0. 002* κα*ιp=0. 006,* respectively*),* while increased LDH at diagnosis correlated with increased risk for progression to myelofibrosis (p=0. 04)**. Conclusions.** T is characterized by an extremely favorable prognosis. Patients with lower hemoglobin levels and lower platelet counts present thrombotic events more frequently, while males and patients with leukocytosis are characterized by an inferior overall survival. Finally JAK2V617F(+) patients differ from JAK2V617F(-) ones regarding their hematological profile and mimic polycythaemia vera patients.

1611

ORIGINAL TOOL TO IDENTIFY OVERLAPPING MPN PHENOTYPES: TRANSITION INDEX

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Background. ET, PV and PMF are chronic Ph- myeloproliferative neoplasms (MPNs) characterized by the presence of the acquired mutation in JAK2 tyrosine kinase gene and by excessive production of mature hematopoietic cells. WHO 2008 diagnostic criteria precisely define these diseases, although there are some overlapping features with different clinical outcome that are not considered in WHO definition. **Aims.** As pure ET, ET with the PV-like phenotype, prefibrotic stage, and early PMF could be considered as a continuum, it might be useful to distinguish different cluster of patients and attribute a different prognostic score to each clinical entity. **Methods.** We analyzed 197 ET patients (diagnosis according to WHO 2008) using a multidimensional approach, the *Multiple Correspondence Analysis* (MCA) and the Automatic Classification (CA). MCA generates a Factorial Plain providing a flat view of the relationship between qualitative variables. 'Synthesis factors' independent of each other, were identified by a linear combination of initial variables. CA identifies criteria to define homogeneous groups of ET patients through graphical representation using dendrograms. The initial selected variables are the following: BM reticulin grade; clustering of megakaryocytes (MK) and/or atypical MK; WBC count (<7, 7-8. 5, 8. 5-10, 10-12, >12 x 10^3/mm^3); and dacryocytes and/or leukoerythroblastosis. The analysis has led to new variables, which have been determined by linear combinations of the initials. Among them we considered the one that better describes our initial sample. This new variable is a continuous variable, which called Transition Index (TI). This term was also inspired to define an overlapping tendency of different ET phenotypes. Thereafter, hierarchical cluster analysis has generated a hierarchy of clusters with different TI, represented as a dendrogram. We identified three main clusters: low TI (n=69), intermediate TI (n=112), high TI (n=16). **Results.** TI correlates with the percentage of patients harboring the $JAK2^{V617F}$ mutation, spleen volume at the time of diagnosis and its enlargement at follow-up, CD34+ cells count in the peripheral blood, iron stores, LDH, beta2-microglobulin and low Hb levels. No correlation was found between TI and response to treatment. Furthermore, a higher TI is associated with a greater frequency of thrombotic events. **Conclusions.** TI could represent an instrument to evaluate the degree of overlapping of a con-

tinuum of not well defined Ph- MPNs. This index identifies patients with different clinical phenotypes, most similar to PV or PMF, who do not match all the criteria for these diagnoses. High value of TI correlates with a worse prognosis and could suggest an intensive follow up or the need to adopt new therapeutic strategies.

1612

CLINICAL FOLLOW-UP OF PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS PRESENTING SKIN ULCERS DURING TREATMENT WITH HYDROXYUREA

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Background. Hydroxyurea (HU) is widely employed in the treatment of Myeloproliferative Neoplasms (MPN); however, painful skin ulcers are a limiting toxicity during HU treatment in >5% of patients. **Aims and Methods.** To evaluate the clinical impact of such complication on the outcome of these patients, we retrospectively revised 1001 cases (M/F 437/564, median age 65. 6 years, IR 55. 6 - 73. 7) with MPN consecutively diagnosed at 6 Centers in Rome who received HU treatment during the course of disease. **Results.** There were 537 patients with Essential Thrombocythemia (ET), 336 with Polycythemia Vera (PV), 102 with Primary Myelofibrosis (PMF) and 26 with unclassifiable Chronic Myeloproliferative Disorders (CMPD-u); 863 patients (86. 2%) received HU as 1st line treatment while 138 (13. 8%) as 2nd or 3rd line treatment. On the whole, 71 patients (7. 1%) developed painful skin ulcers after a median period of 54. 1 months (IR 27. 7 - 97. 6) from HU start; as concerns the site, in 56/71 patients (78. 8%) skin ulcers were located in the perimalleolar area while in the remaining 15 patients in other skin areas (localized to the head or to the extremities in 8 and 7 patients, respectively). When the skin ulcers occurred, HU treatment was continued at the same dosage in 11 patients (15. 4%), was reduced in 13 patients (18. 4%) and temporarily interrupted in 11 patients (15. 4%): the remaining 36 patients (50. 8%) needed a permanent drug discontinuation. Among these latter patients, pipobroman was started in 20 patients, anagrelide in 5, alpha-interferon in 3, melphalan in 3; in addition, no further treatment was given in 1 patient and 4 patients were lost to follow-up. As to ulcer resolution, 11/71 patients were not evaluable (2 too early, 9 lost to follow-up). Among the 60 evaluable patients, after a median period of 6. 3 months (IR 3. 6 - 11. 3) from the onset of the skin ulcers, 43 patients (71. 6%) had a complete resolution and 17 patients (28. 4%) had an improvement without complete resolution. The incidence of 2nd neoplasia [3/71 (4. 2%) patients with skin ulcers vs 73/930 (7. 8%) patients without skin ulcers] and blastic phase evolution [2/71 (2. 8%) patients with skin ulcers vs 41/930 (4. 4%) patients without skin ulcers] were not increased after the skin ulcer occurrence. After a median period from skin toxicity of 30. 7 months (IR 14. 3 - 63. 6), 9 patients were lost to follow-up, 11 patients died and 51 patients are still alive. **Conclusions.** Painful skin ulcers during HU treatment are a relatively common complication in MPN patients, require HU discontinuation in > 50% of cases and in a sizable rate of patients there is only a partial healing of skin lesion: however, this complication and the requested treatment changes do not seem to impact on the subsequent clinical follow-up of MPN patients.

1613

CLINICAL AND BIOLOGICAL FEATURES OF POLYCYTHEMIA VERA (PV) IN YOUNGER PATIENTS: A RETROSPECTIVE ANALYSIS ON 600 PATIENTS FOLLOWED IN THE LAZIO REGION DURING 40 YEARS

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Background. Less than 20% of Policythemia Vera (PV) patients are below 50 years of age at diagnosis and there are very few reports addressing this subset. **Aims.** To compare the clinical and biological features of younger (≤ 50 years) PV patients with adult PV patients (> 50 years). **Methods.** We evaluated retrospectively a cohort of 600 $JAK2^{V617F}$ positive PV patients (median age 61. 4, M/F 346/254) consecutively diagnosed at 11 hematologic Units in the Lazio region from 6/1969 to 1/2011; of them, 123 patients (M/F 75/48) aged ≤ 50 years (group A) and 472 (M/F 268/204) aged >50 years (group B). Diagnosis was made according to the criteria accepted at the time of patients diagnosis. **Results.** Hematological characteristics at onset (mean Ht, WBC and PLT counts) as well as gender distribution did not differ significantly between the two age subgroups; however, mean Hb was higher in younger patients as compared to adult patients (p=0. 04). There was a statistically significant difference in the previous thrombotic events (18/123 in group A vs 107/472 in group B, p=0. 04); in particular, splancnic vein thromboses (SVT) were more frequent in group A as compared to group B (p < 0. 05). On the contrary, thrombotic events during the course of disease did not differ between the two groups (18/123 in group A vs 64/472 in group B, p=0. 8) During the follow-up, 38/123 younger patients (30. 1%) needed to start a PLT-lowering treatment compared to 218/472 patients (46. 2%) aged > 50 years (p<0. 05): in addition, a treatment with phlebotomies only was more commonly employed in younger patients (105/123 vs 361/472, p=0. 02). There was no difference between the 2 groups as to evolution in myelofibrotic/leukemic phase (p=0. 3). **Conclusions.** Younger patients with PV seem to have a clinical presentation at diagnosis and a clinical course similar to patients aged > 50 years, apart from a higher incidence of SVT in the anamnesis.

1614

IMPROVING SURVIVAL TRENDS IN PRIMARY MYELOFIBROSIS: AN INTERNATIONAL STUDY

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Background. Despite the lack of major improvements in the treatment of primary myelofibrosis (PMF), there are recent indications that survival of patients might have increased over the years. **Aims.** The purpose of the present study was to ascertain whether survival prolongation has actually occurred in PMF and to identify which groups of patients have benefited from such improved survival. **Methods.** 802 patients diagnosed with PMF in four European countries were compared for presenting features and survival according to the diagnostic periods 1980-1995 (n= 434) and 1996-2007 (n= 368). Relative survival and PMF-attributable mortality were calculated by adjusting the patients' actuarial survival for that expected in a subset of the general population matched to the patients by age, sex, country of origin, and calendar year of diagnosis. Comparisons between groups of patients were done by Poisson regression. **Results.** Patients diagnosed between 1996 and 2007 had more often constitutional symptoms (31% versus 23%), but lower incidence of marked anemia (31% versus 39%), leukocytosis > 25 x 10^9/L (9% versus 13%), and blood blasts (27% versus 33%). The distribution of risk groups defined by the IPSS was comparable between the two periods. Median survival was 4. 6 years (95% CI: 4. 0-5. 1) for patients from 1980-1995 and 6. 5 years (95% CI: 5. 5-7. 4) for those from 1996-2007 (p< 0. 0001). Patients diagnosed in the more recent period showed improved relative survival, especially women, patients

younger than 65 years, and those with low or intermediate-1 risk disease. Incidence rates of PMF-attributable mortality at 5 and 10 years were significantly lower in the second period; this reduction in disease-specific mortality occurred across all patient subgroups, except in patients within the intermediate-2 or high risk categories of the IPSS. **Conclusions.** Survival of PMF is improving, except in the patients within the poor risk categories. This observation must be taken into account at the time of evaluating the survival impact of newer therapies for PMF, which are currently being tested in these patient subpopulations.

1615

IDIOPATHIC THROMBOCYTOPENIA (ITP)-LIKE PRESENTATION IN CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) AND OUTCOME

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Background. Chronic myelomonocytic leukemia (CMML) is one of the myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) overlap as per 2008 World Health organization (WHO). Cytopenias are commonly found, however, sole thrombocytopenia in the setting of normal hemoglobin raises the suspicion of immune causes. Autoimmunity is seen in patients with MDS and MPN, where its clinical significance is unknown. **Aims.** Describe the characteristics and outcome of patients diagnosed with idiopathic thrombocytopenia (ITP)-like presentation in CMML. **Methods.** A retrospective study of CMML patients diagnosed at Mayo Clinic between 3/1996-8/2011. An IRB protocol was approved. Patients were identified if they had ITP in their history, or have platelets < 50K in the setting of normal hemoglobin and no other possible explaining cause. Information was obtained from chart review and included demographics, autoimmune antibodies work-up, WHO-defined CMML as per pathology review at Mayo clinic. Survival curves and estimates were obtained using Kaplan-Meier through JMP software version 9. 0. **Results.** All 289 patients diagnosed with CMML at Mayo Clinic were screened where 10 (3. 5%) patients had ITP (diagnosed by treating hematologists). Median Age at diagnosis was 74 (range 39-81), WBC 7. 6 (2. 4-33. 4), hemoglobin 12 (10. 5-14. 8), platelets 53 (11-129), LDH 198 (111-424) with 7 being males (70%). All ten patients were classified as CMML1 and had diploid karyotype (but 1 patient had complex cytogenetics). Three of 10 patients had JAK2V617F mutation done, 2 (67%) of which were positive. Two of 8 available patients had splenomegaly, 5 had splenectomy (due to a prior ITP diagnosis) and 1 had no splenomegaly. Interestingly, ITP was diagnosed in 5/10 (50%) patients prior (median time to CMML 599 days) to CMML, 2 after of CMML (median time to ITP 163 days), while 3 patients were found to have thrombocytopenia and CMML simultaneously. Two of 10 (20%) cases were heralded by either MDS or MPN (925, 1399 days). Positive autoimmune antibodies were found in 4/8 (50%) patients (ANA), 1/6 (17%) patients (rheumatoid factor), and 0/1 patients (ANCA); while one patient had sweet syndrome. One patient had psoriasis and one had giant cell arteritis; no patient developed clinical systemic lupus or rheumatoid arthritis. All patients received prednisone for the management of ITP; 3 of which responded (30 %). Three patients received rituximab; 2 (67%) responded. Response to splenectomy was documented in 2/5 (40%) of patients. Three patients were given hypomethylating agents (2 decitabine, 1 azacytidine) for the management of CMML, none of which one achieved complete remission. Only one patient progressed to AML (296 days). Median overall survival was 1131 days in the ITP group compared to 503 days in the rest of the CMML group (p=0. 13 Wilcoxon). **Conclusions.** ITP-like presentation is an infrequent phenomenon in CMML patients and can either precede, follow or coincide with the diagnosis of CMML. It is usually present in low risk CMML and is suspected if no anemia coexisted. Two thirds of our patients did not respond to steroids and had to get splenectomy or second line agent rituximab. ITP-like presentation did not affect overall survival for CMML patients.

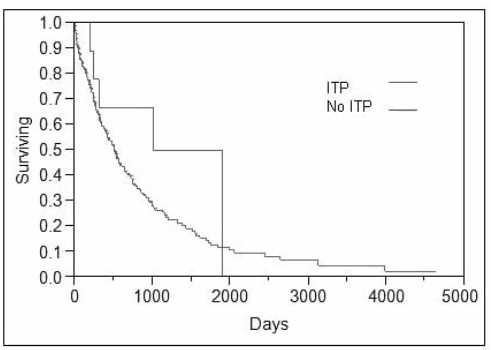

Figure 1. Overall survival.

1616

DIAGNOSTIC FEATURES AND OUTCOME OF PATIENTS WITH MYELOFIBROSIS: IMPACT OF JAK2V617F MUTATION

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Background. Myelofibrosis (MF) is a Philadelphia-negative chronic myeloproliferative disorder characterized by bone marrow fibrosis, progressive anemia and splenomegaly. Recently, JAK2V617F mutation has been detected in about 50% of patients with MF but real impact between JAK2 mutational status, diagnostic features and outcome is not completely known. **Aims and Methods.** We analysed 180 patients referred to our center with diagnosis of Idiopathic MF, prefibrotic MF, post-Polycythemia Vera (post-PV) and post-Essential Thrombocythemia (post-ET) MF, by WHO classification. The diagnosis of MF was made between 1985 and 2011 years. The aim of the study was to evaluate diagnostic features and therapeutic approaches of MF patients, and their outcome in term of thrombotic risk and overall survival (OS), with particular attention to impact of JAK2V617F mutation. T Student and χ^2 tests were used for statistical analysis. **Results.** The median age at diagnosis was 65 years (range 32-89 years). 110 patients (61%) presented Idiopathic MF, 6 (3. 5%) pre-MF, 21 (12%) post-PV and 38 (21%) post-ET MF. JAK2V617F mutational status was tested by qualitative molecular analysis in 152 pts: 77 patients (43%) resulted JAK2 positive (JAK2+). All 6 pre-MFI and 81% of post-PV MF were JAK2 mutatedAt time of diagnosis the median value of WBC count, Hb level and PLT count were 10. 3x10(e)9/L, 11. 8 g/dl and 373x10(e)9/L, respectively. A statistical difference between JAK2+ and negative (WT) group in term of Hb level (14 vs 11 gr/dl, p=0,0005) and WBC count (10 vs 8,5 x10(e)9/L, p=0,048) were observed. Thrombotic and hemorrhagic events, at diagnosis or during follow-up, occurred in 28 (15. 5%) and 12 patients (7%) respect to 16 (9%) and 8 patients (4. 5%), respectively. The incidence of thrombotic complications at diagnosis was greater in JAK2+ patients respect to JAK2WT (24% vs 13%, p=0,05)Regarding to therapeutic approaches, 69 patients (38%) received no therapy and 90 patients (50%) received myelosuppressive agents, with most necessity of cytoreduction for JAK2WT group (49% vs 22%, p=0,0001). 38 patients (21%) received blood transfusions with higher transfusion requirement in JAK2WT patients (42% vs 2,6% p=0,0001)According to Cervantes score, 10 patients (5%) were assigned to high risk (HR), 34 (19%) to intermediate risk-2 (IR-2), 70 (39%) to intermediate risk-1(IR-1), and 40 (22%) to low risk (LR) group. We observed a significant higher prevalence of JAK2V617F mutation in LR group respect to JAK2WT group (LR 52,5% vs 30%, p=0,001)83 patients (46%) are actually alive, after a median follow-up of 26 months. OS at 5 years was poorer for JAK2+ respect to JAK2WT patients (28,5% vs 40%, p=0,055). **Conclusions.** In our cohort of 180 MF patients, JAK2V617F mutation is associated with specific features at presentation and during follow-up, and correlate with clinical outcome. JAK2+ patients present at diagnosis higher Hb level and are less likely to require blood transfusion during follow-up. Also JAK2+ patients receive less cytoreduction and we note a trend for poorer outcome in JAK2+ patients. The incidence of thrombotic complications, instead, is greater in JAK2+ group.

1617

MYELOPROLIFERATIVE NEOPLASMS: CLINICAL PHENOTYPES, JAK2 MUTATIONAL STATUS AND ANGIOGENIC ACTIVITY

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Background. The Philadelphia-negative myeloproliferative neoplasms (MPNs) overlap in their clinical and biological features. A different JAK2 allele burden in these entities has long been noticed. Some data favour the hypothesis that the level of JAK2 (V617F) expression influences the MPN phenotype. We presume that there is a correlation between the JAK2 mutational status and the clinical phenotypes well as with the angiogenesis in the bone marrow. **Aims.** This study was aimed at analyzing newly diagnosed patients with MPNs with regard to some clinical features, risk stratification, $JAK2^{V617F}$ mutational status, bone marrow (BM) fibrosis and angiogenesis. **Methods.** Apart from the standard methods used routinely for the assessing of the BM features (H&E, Gomori), an anti-CD34 antibody was utilized as an endothelial marker to visualize the micro vessels (MV). The micro-vessel density (MVD) was assessed by counting all MV in 5 hot spots on a magnification 10x40. $JAK2^{V617F}$ expression was determined by RT-PCR. SPSS 17 was used for the statistical analysis. **Results.** Totally of 97 patients were analyzed (PV, n=47; IMF, n=31 and ET,

n=19), in 36 of them we estimated the MVD as well. The distribution according to the JAK2 mutational status was: wild-type(n=25), heterozygous type (n=43), homozygous type (n=29). Significantly increased MVD was established in the patients with PV and IMF comparing to ET patients, most prominently in IMF (p<0. 001). Considerably increased neovascularization was determined in $JAK2^{V617F}$ homozygous cases comparing to the wild type, with a borderline statistical significance (r=0. 34; p<0. 05). There was a statistically significant prevalence of grade 2/3 BM fibrosis in JAK2 positive MPN patients. In PV group, the JAK2 positivity (regardless of the mutational burden) correlated with the risk stratification (p<0. 01) and the homozygosity correlated with the splenomegaly (p<0. 001) and more prominent constitutional symptoms (p<0. 001). 74% of the IMF patients were found JAK2 positive and the heterozygocity was associated with significantly higher hemoglobin level (erythroid phenotype) and marked constitutional symptoms. Homozygous IMF patients showed significant leukocytosis (p<0. 01), thrombocytosis and tendency to an anaemia. In ET group, JAK2 positive patients (no homozygous patients were found) were associated with an erythroid phenotype and a tendency to a splenomegaly. No correlation was established between the JAK2 mutational status and the risk stratification in IMF and ET groups. **Conclusions.** Here, we confirm that the JAK2 mutational burden in IMF and ET correlates with different clinical phenotypes (erythroid, thrombocytemic) and could explain the phenotypic mimicry among these MPNs. As a common pathophysiological process it could not contribute to the distinction among the overlapping features in PV, ET and IMF patients. The other WHO diagnostic criteria should be used always to determine the most relevant diagnosis and thus the prognosis of the patient. The assessment of the MVD is an easily approachable method for a routine use and the level of an increased angiogenic activity could assist in distinguishing the disputable cases among the MPNs. Therefore, this process is worthy of a further investigation in MPN patients in the context of the JAK2 mutational status.

1618

CLINICAL SIGNIFICANCE OF JAK2 MUTATION IN PATIENTS WITH CHRONIC MPN AND EVALUATION OF IT AS A RISK FACTOR FOR HEMORRAGIC COMPLICATION IN THESE DISORDERS

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Background. JAK2 mutation is recurrent in myeloproliferative neoplasms (MPNs). It has been recognized as a thrombotic risk factor in MPNs, probably due to an increased myeloid proliferation and white blood cells (WBC) activation, but only few data are available about the effect of JAK2(V617F) on hemorrhagic risk. **Aims.** In our study we investigate the frequency of JAK2V617F mutation in a lot of 194 patients with MPNs diagnosed in our clinique and we evaluate the influence of the mutational status on hemorrhagic complication. **Methods.** JAK2V617F mutation was screened by allele-specific polymerase chain reaction (AS-PCR). We retrospectively analysed laboratory and clinical findings of 194 consecutive patients with MPNs to evaluate possible relationships between thrombosis, abnormal bleeding, peripheral blood count and overexpression of JAK2(V617F) mutational status. **Results.** JAK2V617F mutation was detected in 124 of the 194 patients with MPN. The frequency was similar among ET, IMF and MPD-U (P > 0. 05), but it was significantly lower than that in PV (P < 0. 05). The presence of JAK2V617F was found to be significantly correlative with middle age at diagnosis (P < 0. 01) and with higher hemoglobin levels and higher leukocyte counts (P < 0. 05). Significant difference was found in complication of vascular events between JAK2V617 positive and negative patients (P < 0. 05). Cytogenetic analysis was performed in 62 patients from the entire lot of 194. Among the subgroup with normal karyotype, JAK2V617F mutation was detected in 30% patients as compared with 90% of the patients from the subgroup with karyotypic abnormalities (which were 70% from those to whom we performed cytogenetic analysis). We also found an association between JAK2(V617F) mutation and thrombotic events before or at diagnosis (p<0. 003, OR=4. 44, 95% CI=1. 74-12. 4); no statistical correlation between the median value of JAK2(V617F) burden and an increased risk of thrombosis (p=0. 4, 95% CI= -22. 8-10. 4); significant relationships between mutated status and higher haematocrit, high WBC count and low platelet count. Moreover, the presence of the JAK2(V617F) mutation and a WBC count greater than 9. 2 x 10(9)/L were found to be independent factors related to thrombotic complications in multivariable analysis. The prognostic impact of JAK2 mutation status and WBC count on thrombosis was evaluated in the whole cohort. Finally, wild-type JAK2 was associated with a higher haemorrhagic risk in univariate analysis but only a platelet count greater than 1,022 x 10(9)/L was associated with an increased risk of bleeding in the multivariable analysis. **Conclusions.** The presence of JAK2V617F in MPNs is associated with the disease development. There is a correlation between JAK2V617F mutation in MPN and advanced age, higher leukocyte counts, hemoglobin level and vascular events. Our data confirm the role of both JAK2(V617F) as factor associated with an increased risk of thrombosis at the diagnosis and during follow-up in no treated patients. Moreover a WBC count over 9,2 x 10(9)/L1 was also strictly associated to an increased risk of thrombosis. Regarding bleedings, our statistical analysis allows to exclude the mutation protective role on haemorrhage.

1619

THE OXIDATIVE STATUS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background. Essential Thrombocythemia (ET), one of the chronic myeloproliferative disorders, is a clonal disease in which thrombotic and hemorrhagic complications can be seen. Oxidative stress parameters are shown to be increased in patients of hemodialysis, lung cancer and cases of pulmonary embolism with ischemia. Yet, to our knowledge, no studies conducted investigating the oxidative status of the patients of ET. **Aims.** In the present study, we have planned to determine the serum levels oxidative parameters like the total oxidative status (TOS), total antioxidant status (TAS), oxidative stress index (OSİ), ischemia-modified albumin (IMA) and malondialdehyde (MDA) in ET patients. **Methods.** 43 ET patients (20 male, 23 female) and 20 healthy volunteers (control group) with similar age are enrolled in the study. Oxidative stress parameters (TOS, OSİ, TAS, MDA, İMA) are compared between control and patients at the time of diagnosis. The same parameters of the patients at the time of diagnosis and 6 months after the therapy were also compared. **Results.** Compared to control, oxidative status parameters like TOS, OSI and MDA are found to be significantly higher in patient group. TAS levels were significantly reduced in patients. IMA results were similar in patient and control group. After the therapy, OSI and MDA values were significantly reduced in patient group compared to the values before treatment. Following the treatment, TAS values showed a statistically insignificant increment. Similarly, TOS and IMA levels were found to be reduced but these changes did not reach to significance level. **Conclusions.** Our findings reveal that oxidative stress parameters increased but the compensative antioxidant capacity was significantly reduced in ET patients compared to healthy controls. The alterations observed in oxidative stress parameters of ET patients might be related to thromboembolic events.

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CLINICAL, HISTOLOGICAL AND LABORATORY ASPECTS IN PATIENTS WITH MPN PH- CARRYING TRISOMY 9

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Background. The Myeloproliferative neoplasms (MPN) comprise several clonal haematological diseases that are thought to arise from a transformation in a hematopoietic stem cell. The three main Ph-negatives are Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Idiopathic Myelofibrosis (IMF). The main clinical features of these diseases are the overproduction of mature functional blood cells and long clinical courses. Trisomy 9 has been described in several haematological disorders, representing the third most frequent chromosome abnormality in Ph- neoplasms after 20q deletion of and trisomy 8. **Aims.** In this study we have analysed clinical, histological and laboratory features including *JAK2*V617F allele burden in MPN patients carrying trisomy 9, to understand if this cytogenetic anomaly is associated with some particular aspects. **Methods.** Cytogenetic analysis was performed on 325 MPN patients followed by our institution by QFQ-banding on unstimulated 24 hour cultured

bone marrow. *JAK2*V617F allele burden was measured on genomic DNA from granulocytes using JAK2 MutaQuant® Kit (Ipsogen). **Results.** Trisomy 9 was found in 13 cases (9 PV and 4 ET). In 10 of them it was the only chromosomic anomaly found, in one case it was associated with trisomy 8 and in the last two cases in complex karyotypes. Patients were regularly followed-up for a period of 10. 6 years (range 1-23 years). The median age of diagnosis was 62 years and M:F ratio was 1,2:1. Laboratory data revealed a mean Hb level of 16,7 g/dl, WBC count of 9657/mmc, while mean Plt count was 690. 000/mmc. Erythropoietin serum level was reduced in all patients and *JAK2*V617F mutation was found in almost all patients (mean allele burden 47,1%). Morphological analysis revealed that bone marrow cellularity was increased in all patients carrying trisomy 9; in particular, both erythropoiesis and granulopoiesis were increased with left shifting. In addition, in these patients there were more pleomorphism of the megakaryocytes, defined by the presence of small to giant megakaryocytes grouped together, more nuclear hyperlobulation, maturation defects, bulbous and naked nuclei. It is worth noting that Hb levels at diagnosis in the 4 ET patients were higher than normal but not enough for PV diagnosis; three out of them later evolved to PV. **Conclusions.** The above data demonstrate that trisomy 9 in our study is more frequent in PV. Patients with ET carrying this anomaly showed clinical, histological and laboratory characteristics similar to PV with a risk of evolving into overt PV, which is a relatively rare event. . Remarkably, all ET patients with trisomy 9 were positive for V617F mutation of *JAK2* gene, which is mapped on the short arm of chromosome 9.

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ANALYSES OF RISK FACTORS PREDICTING THE THROMBOTIC COMPLICATIONS IN LONG-TERM FOLLOW UP OF 207 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background. The clinical course of essential thrombocythemia (ET) is marked by a higher incidence of thrombotic complications, which present the main cause of morbidity and mortality. Major predictors for thrombosis are age over 60 years and previous thrombosis. Myelosuppressive drugs can reduce the rate of thrombosis, but there is concern that their use raises the risk of transformation into acute leukemia. To solve this dilemma, the risk-oriented treatment strategy is recommended. **Aims.** We investigated the clinical and laboratory parameters associated with the risk of the occurrence of thrombotic events to identifying the subgroups of patients who will have the benefit from cytoreductive therapy. **Methods.** The study involved 207 consecutive ET pts who were diagnosed according the WHO criteria (with information of bone marrow biopsy at diagnosis) and followed from January 2000 to December 2010 at Clinic for Hematology, Clinical Center of Serbia, Belgrade. The median follow up of the study cohort was 64 months. **Results.** There were 137 females and 70 males with median age 57 years (range 18-85); median Plt count was 1067x10^9/L (range 497-3600); median WBC count 10. 5x10^9/L (range 5. 4-22. 8); median Hb 13. 9 mg/L (range 9-180); symptoms of ET had 104/171 (61%) mostly paresthesia and headache; history of hemorrhage had 4 pts (2%). Previous thrombosis had 29 pts (14%), 22 pts experienced arterial, 5 venous and 2 both. During the follow up, 11pts (5. 3%) developed thrombosis (7 arterial and 4 venous): 4/25 pts with a previous history of thrombosis and 7/171 without a history of thrombosis (p=0. 028). Median time of occurrence thrombotic event was 45months (range 6-130 months) after the diagnosis of ET. The thrombotic events at onset of disease were significantly related to age over 60 years (p=0. 02) but no relationship was found with number of leukocyte, platelets, gender and JAK2V617F mutation. Patients were divided into subgroups according to the presence of the four analyzed risk factors including age (over 60 years), count of Le (>10x10^9/L), count of Plt (>1500x10^9/L), and the presence at least one of the cardiovascular risk factor (tobacco use, hypertension, diabetes). The rate of thrombosis during the follow up was significantly higher in a subgroups of patients with two or more risk factors than in subgroups of patients without any or with presence of one risk factor (p=0. 001). **Conclusions.** Previous thrombosis and platelet count, generally accepted as very important risk factors for thrombosis were not associated with an increased risk in our group. The rate of thrombosis at the onset of disease has been confirmed to be related to the age over 60 years. Moreover, a better prediction of thrombotic risk was found in overview of the combination of risk factors. Patients with two or more analysed factors are at the high risk to develop thrombosis.

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CLINICAL CHARACTERISTICS OF JAPANESE ADULTS WITH INTOLERANT/REFRACTORY HIGH-RISK ESSENTIAL THROMBOCYTHEMIA - BASELINE DATA FROM A PHASE III, MULTICENTER, EFFICACY AND SAFETY STUDY OF ANAGRELIDE

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Background. Essential thrombocythemia (ET) is a myeloproliferative disorder associated with an increased risk of thrombohemorrhagic complications. A previous study reported significant clinical differences between patients with ET in Caucasian and Japanese populations, with a lower incidence of thrombosis in the latter[1]. Clinical characteristics have not previously been described in high-risk patients (>60 years of age; history of thrombosis; platelet count >1000x10^9/L) with ET who are intolerant or refractory to their existing cytoreductive therapy in a Japanese population. Ranimustine is currently the only licensed treatment for patients with ET in Japan, however, a number of cytoreductive therapies are available. **Aims.** The objective of this study is to assess the clinical characteristics of high-risk patients with ET who are intolerant or refractory to currently available cytoreductive therapy in Japan. **Methods.** High-risk patients aged ≥20 years with ET, diagnosed according to WHO criteria, who were intolerant or refractory to their existing cytoreductive therapy, were enrolled into the study, SPD422-308 (NCT01214915). Informed consent was obtained and baseline characteristics were recorded. Patients subsequently received a starting dosage of anagrelide (1mg/day), administered orally as 0. 5mg twice daily. After 1 week the dose was titrated to a patient-specific optimal dosage required to achieve a response. Patients are planned to receive treatment for 12 months. This study is sponsored by Shire Pharmaceuticals. **Results.** A total of 53 patients, 56. 6% females (n=30) and 43. 4% males (n=23), assessed to be intolerant (64. 2%, n=34) or refractory (35. 8%, n=19) to their existing cytoreductive treatment, were enrolled into the study. The median age at screening in the intolerant and refractory groups was 67 years (range, 36-86) and 61 years (range, 36-71), respectively. The median time from date of diagnosis until study enrollment was 6. 9 years (range, 0. 1-29. 1) and 7. 1 years (range, 0. 2-22. 2) for intolerant and refractory groups, respectively. Notably, four patients received cytoreductive therapy prior to being formally diagnosed. The median platelet count at baseline was 826. 5x10^9/L (range, 285-2130) and 1030x10^9/L (range, 601-2518), respectively. Baseline hematological values comparing patients who were intolerant or refractory to their existing cytoreductive therapy are summarized in Table 1.

Table 1. Hematological values at baseline comparing Japanese high-risk patients with ET who were intolerant or refractory to their existing cytoreductive treatment.

	Intolerant patients	Refractory patients
Number of patients (%)	34 (64.2)	19 (35.8)
Platelets (10^9/L) Median (range)	826.5 (285–2130)	1030 (601–2518)
Hb (g/dL) Median (range)	119 (69–150)	127 (86–159)
Hct (V/V) Median (range)	0.37 (0.21–0.51)	0.40 (0.26–0.50)
WBC (10^9/L) Median (range)	7.3 (2.4–29.2)	6.0 (2.7–27.4)

Hb – Hemoglobin; Hct – Hematocrit; WBC – White blood cells

The most common cytoreductive therapy that patients were refractory or intolerant to, prior to the first dose of study treatment, was hydroxycarbamide (96. 2%, n=51). **Summary and Conclusions**. This study provides important baseline data on a previously undescribed population of patients with ET, who are intolerant or refractory to their existing cytoreductive therapy. As expected, patients considered to be refractory to their existing cytoreductive treatment were observed to have a higher platelet count at baseline, compared with patients who were intolerant to their existing cytoreductive therapy. However, for both groups the median platelet count was higher than the recommended target of <400-600x10^9/L [2]. This study highlights the need for alternative treatment options in both refractory and intolerant high-risk patients with ET in Japan.

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CYTOREDUCTIVE COMBINATION THERAPY IN ESSENTIAL THROMBOCYTHEMIA: PRELIMINARY REPORT OF THE REGISTRO ITALIANO TROMBOCITEMIA (RIT)

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Background. The cytoreductive agents Hydroxyurea (HU), Anagrelide (ANA), and Interferon alpha (IFN) in ET patients are not rarely discontinued due to toxicity/side effects or inefficacy. Since HU, ANA, and IFN have different activity and toxicity patterns, the combined use of two drugs may result useful to overcome the inadequacy of a single drug. OBJECTIVETo evaluate the role of the cytoreductive combination therapy in the clinical management of ET patients. **Methods.** The RIT includes 2550 ET patients (PVSG or WHO criteria). A data analysis has been performed by distinguishing combinations on the basis of the first (baseline) drug and of the second (added) drug: HU+ANA, ANA+HU; IFN+ANA, ANA+IFN; HU+IFN, IFN+HU. Frequency, rate and reasons of discontinuation, and duration of the different combination therapies are considered. **Results.** A total of 118 (4. 6%) ET patients of the RIT received a combination therapy between March 1993 and May 2011, at median distance of 3 years from diagnosis. The patients, 49 males and 69 females, had at diagnosis: median age 48 years (range 5-79), median PLT count 979 x 10^9/L (range 490-2000), JAK2 V617F mutation (52%), disease related symptoms (39%), CV risk factors (43%). The patients performed globally 129 courses of combination therapy: HU+ANA (n 50), ANA+HU (n 20); IFN+ANA (n 19), ANA+IFN (n 5); HU+IFN (n 29), IFN+HU (n 6). In these 129 courses the baseline drug was represented by HU in 79 cases, ANA in 25 cases, and IFN in 25 cases, while the added drug was ANA in 69 cases, IFN in 34 cases, and HU in 26 cases. The combination therapy was interrupted globally in 73 out of 129 courses (57%), with lower frequency in the HU+ANA or ANA+HU courses (47%, 33 out of 70) and higher frequency in the other combination courses (68%, 40 out of 59). These 73 interruptions were due to HU withdrawal (27 courses, 37%), IFN withdrawal (23 courses, 32%), ANA withdrawal (18 courses, 25%), other reasons (5 courses, 6%). The most frequent reasons of drug withdrawal were: for HU toxicity/side effects (n 11), and inefficacy (n 6); for IFN toxicity/side effects (n 8), and inefficacy (n 4); for ANA toxicity/side effects (n 10). The rate of the baseline drug withdrawal was 20% for ANA (5/25 courses), 32% for IFN (8/25 courses), and 33% for HU (26/79 courses). The median duration of combination therapy was 9 months (range 0. 5-86) for all 129 courses, 12 months (range 0. 5-86) for the 56 ongoing courses, and 6 months (range 0. 5-68) for the 73 interrupted courses. The median duration was longer (15 months) for the HU+ANA or ANA+HU ongoing courses and lower (6 months) for the other ongoing courses. **Conclusions.** A cytoreductive combination therapy was reported in 118 (4. 6%) out of 2550 ET patients of the RIT. The combination HU+ANA or ANA+HU, compared with the other combinations, was the most frequent (54%, 70//129 courses), showed a longer duration (15 vs 6 months), and a lower rate of interruption (47 vs 68%).

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NEUROLOGICAL SYMPTOMS IN ESSENTIAL THROMBOCYTHEMIA: IMPACT OF JAK2V617F MUTATION AND RESPONSE TO THERAPY

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Background. patients with Essential Thrombocythemia (ET) may present a wide spectrum of neurological symptoms (NS) at onset or during the course of disease, only in part resulting from previous thrombotic cerebral events. Sometimes NS may indicate a micro-vascular occlusive event however the risk factors and specific therapeutic strategy have not completely defined yet. **Aims and Methods.** we retrospectively described NS that occurred in 265 ET referred in our center from 1995 to 2011. We separately analysed NS secondary to thrombotic cerebral event (TCE) by the remaining functional ET-related NS, in order to identify clinical and biological features associated to NS and to assess the response of NS to therapy. **Results.** Overall, 86/265 patients (32. 5%) have presented whatever NS; 16/86 patients (18,6%) developed NS after TCE (transient ischemic attack or stroke), principally paresthesias (6) or instability and motor and sensitivity disturbances (10). The remaining 67 patients reported subjective and often transient and fluctuating NS, by frequency order: chronic paresthesias (36), cephalalgia (30), dizziness or hypotension (16), blurry vision (8), tinnitus (5), which we defined as ET-related NS. Six patients presented erythromelalgia as peripheral manifestation. Median age of NS patients was 55 years, with prevalence of female sex; JAK2V617F mutation was evaluated in 65/86 patients with prevalence of JAK2 positive versus negative patients (64,5% vs 35,5%). All patients received anti-platelet agent (low- dose aspirin or similar) at diagnosis; a cytoreductive treatment, instead, was reserved to TCE-patients and selected ET-related NS patients. In most TCE-patients, anti-platelet + cytoreductive treatment didn't lead to complete resolution of NS; in ET-related NS group, 27/64 patients (42%) achieved a complete response after anti-platelet treatment, alone (15) or associated with cytoreduction (12) to normalize platelet count. 58% of ET-related NS patients did not respond to anti-platelet treatment; 8 patients of whom (21,6%) improved NS after addition of citoreductive therapy. Patients not responsive to antiplatelet-only treatment appeared older (59 vs 46 years), but no other differences regarding laboratory features and JAK2 mutational status were demostrated. Therefore, we have subdivided patients with NS in two risk classes according to number of cardiovascular (CV) factors (low: 0; high > 1). Focusing on low risk CV group, we noted that the response rate to anti-platelet treatment was higher in JAK2 positive respect to JAK2 negative patients (50 % vs 25%). Overall, 85% of patients (73/86) with NS are alive with a median follow-up of 52 months (range: 1-312 months). **Conclusions.** We observe that 32,5% of ET-patients present NS. In the 80,7% of them (67/86) NS aren't secondary to TCE, so these are interpreted as ET-related NS. Mostly of these patients are female and seem to present higher prevalence of JAK2V617F mutation. Only 42% of ET-related NS responds to anti-platelet-only treatment¸ in particular in low risk CV group, the rate of response to anti-aggregation is higher in JAK2 positive respect to negative patients. The addition of cytoreductive therapy should be considered in ET-related NS refractory cases, suggesting the involvement of different mechanisms from micro-vascular disturbances, not fully understood yet, in ET-related NS pathogenesis

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UTILITY OF SPLEEN VOLUME EVALUATED WITH ULTRASOUND SCAN IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background. Splenomegaly is a common finding in MPN occurring in about 70% of polycythemia vera (PV), 10% of essential thrombocythemia (ET) and almost all primary myelofibrosis (PMF) since their diagnosis. It has been found that ultrasound scan (US) assessment is a sensitive method to evaluate splenomegaly in MPN. **Aims.** The present study evaluates the efficacy of US in detecting splenomegaly in MPN patients when palpation is not able to recognize splenomegalyMaterials and **Methods.** Spleen size was assessed in 50 patients with MPN (22 PV, 15 ET and 13 PMF, 21 males and 29 females, mean age 63±15 years) diagnosed in agreement with WHO 2008 criteria. We compared palpation (expressed as cm from the costal border) with US assessment. US studies have been performed by a single operator using an Aplio SSA-700A (Toshiba, Japan) instrument with a 2. 5/3. 5-MHz broadband curvilinear probe. Perimeter, longitudinal diameter, and area, defined as the maximum measurements with splenic borders and angles clearly defined, were measured, and volume (in milliliters) was calculated automatically. To obtain normal values of US measurements (longitudinal diameter 9-11 cm, area 27-45 cm^2 and volume 70-160 ml) we used 22 healthy volunteers. We applied c^2

test for categorical variables. **Results.** Splenomegaly was detected by palpation in 25 MPN (50%) and confirmed in all cases by US evaluation. In 14 (56%) out of the remaining 25 patients, US assessment showed increased spleen size. Spleen longitudinal diameter, area and volume were equally efficient in founding splenomegaly. The comparison between spleen palpation and spleen US assessment is summarized in the Table 1. **Conclusions.** We confirm that US assessment is a sensitive method for identifying non palpable splenomegaly in patients with MPN; however, we failed to found a higher sensitivity of spleen volume in detecting splenomegaly compared with measurement of longitudinal diameter and area in contrast to other Authors (Martinelli et al, Blood 2002, 99:4228). While in PMF splenomegaly is well recognized with physical examination, US assessment seems to be particularly useful in documenting small splenomegaly of ET and PV.

Table 1.

	Palpation Pos/neg	US Pos/neg	p
TOT (50)	25/25	40/10	0.003
PV (22)	11/11	19/3	0.02
ET (15)	4/11	9/6	0.03
MF (13)	10/3	12/1	NS

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EVALUATION OF THE DIAGNOSTIC CRITERIA OF POLYCYTAHEMIA VERA IN EVERYDAY CLINICAL PRACTICE: THE RELEVANCE OF JAK2V617F MUTATION

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Background. Polycythaemia vera (PV) belongs to the bcr-abl negative group of myeloproliferative disorders. The introduction of the JAK2V617F mutation, has led to a clearer description of this entity. **Aims.** Aim of the present study is to report the clinical and laboratory findings and the outcome of PV patients from a single center, as well as to evaluate the application of the new diagnostic criteria in everyday clinical practice. **Methods.** The WHO 2008 diagnostic criteria for PV were retrospectively applied in 61 patients diagnosed with PV between 1998 and 2011. Patients were distributed into 3 groups: Group I included patients who fulfilled both major criteria (increased Hb values for gender: Hb≥16. 5g/dL in women and Hb≥18. 5g/dL in men, or increased red cell mass and the presence of JAK2V617F mutation), group II included those who fulfilled the 1st major and two minor criteria (low/low-normal EPO levels and bone marrow biopsy consistent with PV). In this group JAK2V617F mutation was either unavailable or negative. Group III consisted of JAK2V617F(+) patients, who were diagnosed and treated as PV, but did not strictly fulfill the WHO 2008 criteria. **Results.** The median age of our patients was 64 years (29-91), 31 were males and 30 females. At diagnosis, 52% were asymptomatic, 21% had splenomegaly and 16% hepatomegaly. A major thrombotic or bleeding episode was reported at 30% with the most frequent being an arterial thrombosis/ischemia. JAK2V617F mutation was found in 36/38 patients (95%), median values of Ht, Hb, MCV, WBC and PLT were 53. 8%, 17. 3g/dL, 81. 8fl, 9. 050x10^9/L and 575x10^9/L respectively. LDH levels were elevated in 34%, while median serum erythropoietin levels were 6. 65 IU/mL (0-28. 4). Bone marrow biopsy revealed panmyelosis in 55%, fibrosis in 33% with grade 2 fibrosis in 3%, while 12% had an abnormal karyotype. First line treatment was hydroxyurea in 73%, while 24% were treated with phlebotomy only. Median time to the initiation of cytotoxic treatment was 0. 9 months (0-77). Antiplatelet and anticoagulation treatment was administrated in 77% and 7% respectively. The overall response rate to cytotoxic therapy was 95% with 63% complete and 32% partial remission. Median time to response was 2. 4 months. At a median follow up time of 38 months (0. 9-162) 5 deaths were recorded, with a 5- and 10-year overall survival (OS) of 92% and 77% respectively. Age >65 years (p=0. 029), increased LDH (p=0. 026) and the presence of a thrombotic/bleeding event (p=0. 048) at diagnosis were adverse prognostic factors for OS. The comparison of the 3 subgroups revealed statistically significant differences depicted in the Table 1: Subgroup III consists of cases that cannot be objectively classified as PV or essential thrombocythaemia (ET) and might represent the pre-polycythaemic phase of PV. **Conclusions.** Although the new diagnostic criteria of PV make the differential diagnosis between true PV and secondary erythrocytosis clear-cut, they cause difficulty in classifying patients between PV, ET and the pre-polycythaemic phase of PV. The biology of this disease subgroup is not known, while its clinical importance needs to be further elucidated.

Table 1.

	Group I N=23	Group II N=20	Group III N=20	p
Median Ht (%)	55,4	55,1	50,8	0,007
Median Hb (g/dL)	17,7	18,05	16,3	0,000
Median WBC (x10^9/L)	10,43	7,95	11,22	0,05
Median PLT(x10^9/L)	653	423	561	0,01
Median EPO levels (IU/mL)	4,6	12,1	3,1	0,05

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THE OXIDATIVE STATUS IN PATIENTS WITH POLYCYTHEMIA VERA

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Background. Polycythemia Vera (PV) is the most prevalent disease among chronic myeloproliferative disorders. In PV patients, before diagnosis or during follow-up period, life threatening thrombotic events can be observed. Oxidative stress parameters are shown to be increased in patients of hemodialysis, lung cancer and cases of pulmonary embolism with ischemia. However, to our knowledge, studies conducted investigating the oxidative status of the patients of Polycythemia Vera. **Aims.** In the present work, we have investigated the serum levels oxidative parameters like the total oxidative status (TOS), total antioxidant status (TAS), oxidative stress index (OSİ), ischemia-modified albumin (IMA) and malondialdehyde (MDA) in PV patients. **Methods.** 35 PV patients (20 male, 15 female) and 20 healthy volunteers (control group) with similar age are enrolled in the study. Oxidative stress parameters (TOS, OSİ, TAS, MDA, İMA) are compared between control and patients at the time of diagnosis. The same parameters of the patients at the time of diagnosis and 6 months after the therapy were also compared. **Results.** Compared to control, oxidative status parameters like TOS, OSI and MDA are found to be significantly higher in patient group. TAS levels were reduced (statistically not significant) in patients. IMA results were similar in patient and control group. After the therapy, TOS, OSI and MDA values were significantly reduced in patient group compared to the values before treatment. Following the treatment, TAS values showed a statistically insignificant increment. Similarly, IMA levels were found to be reduced but the change was not significant. **Conclusions.** Our findings showed that oxidative stress parameters increased but the compensative antioxidant capacity was significantly reduced in PV patients compared to healthy controls. The alterations observed in oxidative stress parameters of PV patients might be related to thromboembolic event.

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CHARACTERISTICS OF DIAGNOSTIC APPROACHES FOR ERYTHROCYTOSIS OF DIFFERENT GENESIS

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Background We suggest that the common laboratory parameters such as hemoglobin, red blood cell (RBC) number and hematocrit do not always adequately reflect the degree of absolute RBC mass due to possible variations of the plasma volume. Therefore only these values are not completely sufficient for diagnostics of hematological disorder. **Aims.** To investigate the diagnostic value of circulating RBC mass as a possible marker of absolute erythrocytosis in connection with hemoglobin, hematocrit, RBC number in the group of patients with preliminary diagnosis of polycythaemia vera (PV) and to demonstrate the distribution of the investigated patients into diagnostic groups. **Materials and Methods.** We evaluated 61 patients with erythrocytosis before the beginning of treatment, 9 females and 52 males, medium age was 46 years (18-82). Medium level of hemoglobin and hematocrit in females was 171g/l (143-190) and 52% (49-61) respectively. Medium level of hemoglobin and hematocrit for males was 187 g/l(168-196) and 57,8% (49-65), respectively. All samples of venous blood were taken in the morning. Count blood cells (CBC) with differential was performed by Coulter principle on Gen S ("Beckman-Coulter",USA) blood analyzer with preserving agent (ethylene diamine tetra acetate, EDTA). The circulating RBC mass and plasma volume were measured by radionuclide method (Cr-51). The results were calculated in accordance with the patient's body surface area and were interpreted according to the recommendations of International Committee for Standardization in Hematology (ICSH) (Pearson et al. 1995). Range of normal variations of RBC mass and plasma volume for healthy males and females were +/- 25% from the normal individual parameters. **Results.** PV was detected only in 19(31%) of 61 patients. 15 patients refused from further investigation. Among the remaining 46 patients 14 subjects had secondary erythrocytosis, among them 9 had absolute erythrocytosis (hypoxic) and 5 had idiopathic erythrocytosis. Relative ("apparent") erythrocytosis was detected in 13 cases. Measurement of circulating RBC mass allowed us to distinguish the groups of patients with absolute and relative erythrocytosis. We found that hemoglobin level over 185 g/l was confirmed by presence of absolute erythrocytosis only in 50% of males with PV, while 15% of males with secondary erythrocytosis could have an incorrect diagnosis due to "apparent"increased RBC mass. We defined statistically significant (P=0,001) difference of RBC mass in patients with PV and patients with "apparent"erythrocytosis: 166% and 111%, respectively. In patients with PV and absolute erythrocytosis the RBC mass was over the upper normal limit (more than 25%) while the patients with secondary erythrocytosis had RBC mass rate within the normal limits +/- 25%. Average plasma volume measurements in both groups of patients were at normal ranges: 95% и 81%, respectively (P=0,01). **Conclusions.** We consider that the RBC mass and plasma volume measurement is accessible, easy to perform and necessary procedure to distinguish the absolute and "apparent"erythrocytosis. It is important because rather common occurrence of different forms of erythrocytosis and in particular of the "apparent"erythrocytosis should determine different diagnostic approaches according to the specific clinical situation.

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DYSLIPIDEMIA AND MEAN PLATELET VOLUME - RISK FACTORS FOR THROMBOTIC EVENTS IN PATIENTS WITH PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASIAS

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Background. Patients with Philadelphia-negative chronic myeloproliferative neoplasias have an increased thrombotic risk. Increased mean platelet volume is known as an index of platelet activation and of thrombotic risk, like hypercholesterolemia. It is known that leukemic cells have an increased cholesterol metabolism, which they capture intracellular and use it for their own proliferation. **Purpose**: We aimed to analyze changes in lipids and platelets in patients with chronic myeloproliferations with and without a history of thrombotic accidents. **Materials and Methods.** There were included in the study all patients with Ph negative chronic myeloproliferative neoplasias, who were admitted in 2011, in the Hematology Department of Emergency County Clinical Hospital of Sibiu and appear in the electronic filing system ATLAS. We studied: age, gender, diagnosis, platelet count, mean platelet volume, cholesterol, triglyceride, treatment, thrombotic accidents, associated diseases. We note that the blood samples were analyzed in the laboratory, within 2 hours after withdrawing. Patient data were compared. Statistical analysis was performed with the arithmetic mean, standard deviation, Student t test and Pearson test. **Results.** The 50 patients had a mean age 61. 9+/- 13. 8 years. Distribution by gender: 31 women (62%), 19 men (38%). The average number of platelets was 356653. 1+/-259251. 3/mm3, average cholesterol - 173. 4+/-48. 53 mg/dl, average triglyceride 156. 17+/ -78. 36 mg/dl. From the entire group, 18 patients (36%) had a history of thrombotic accidents (8 ischemic stroke, 4 venous thrombosis, 3 myocardial infarctions, two episodes of disseminated intravascular coagulation, a Budd Chiari syndrome and a splenic infarction) (group A). The comparison of the biological data of patients in group A with those with no history of thrombosis (group B), showed that the first have significantly higher values of both triglyceride (193. 06+/-91. 10 mg/dl to 133. 23+/-60. 23 mg /dl) ($p<0.005$) and the mean platelet volume (11. 19+/-0. 84 fl, from 9. 94+/-0. 91fl) ($p<0.00005$) compared with patients without thrombotic history. In patients of group A (not in those of group B) cholesterol values were directly correlated with the triglyceride (r=0. 524) and mean platelet volume was inversely correlated with platelet count (r=-0. 237). So, the higher the mean platelet volume gets the lower the platelet count is and vice versa. In the entire group of 50 patients there was a direct correlation (less intense) between the cholesterol and triglyceride levels (r=0. 314) and between age and cholesterol levels (r=0. 260). **Conclusions.** The mean platelet volume is significantly higher in Ph negative chronic myeloproliferative neoplasia patients with a history of thrombotic accidents, than those without such history. The fact that, mean platelet volume of the patients with thrombotic history correlates inversely with platelet count, can explain why patients with essential thrombocythemia are predisposed to thrombosis at platelet values below 100000/mm^3, compared to those with values above 100000/mm^3, that are predisposed to bleeding. Patients with a history of thrombotic accidents have also higher triglyceride values, which is directly correlated with their cholesterol, which increases thrombotic risk.

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ANAGRELIDE IN THE TREATMENT OF ESSENTIAL THROMBOCYTHAEMIA (ET)

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Background. Essential thrombocythaemia (ET), the most often occurring myeloproliferative disorder is a clonal malignant disorder arising from stem cell. The course of the disease is complicated by some severe thrombotic events and far less commonly by haemorrhagic phenomena. Treatment of ET consist of antiplatelet drugs (e. g. aspirin) and lowering platelet count (hydroxyurea or interferon alpha). Anagrelide is an oral imidazoquinazoline agent which is indicated in Europe for the reduction of elevated platelet counts in at-risk patients with essential thrombocythaemia who are intolerant of or refractory to their current therapy. **Aims.** We evaluated the use of anagrelide in ET patients, previously treated with hydroxyurea or interferon alpha. **Methods.** In our institution we are following 131 patients affected by ET (77 females and 54 males; median age 48 years, range: 27-85) according to PVSG criteria and WHO classification. 75 out of 131 patients were classified as low-risk, 31 as intermediate-risk and 25 as high-risk. The Val617Phe point mutation of Janus Kinase 2 gene (JAK2V617F) was found in 72 patients. **Results.** Anagrelide was used in 27 patients with ET from Jan. 2010 to Jan. 2012. Anagrelide, in the average dose of 2,0 mg (range: 1,0-3,5 mg) reduced platelet count in all patients. Median time of response was 3-4 weeks. Complete remission (platelet count < or = 450x10^9/l) was achieved in 25/27 patients, and only one patient had platelet count slightly above 450x10^9/l (but less than 600 x10^9/l). During the first two months of treatment with anagrelide some mild and transient side effects were noticed, e. g. headache in 7 (47%), fluid retention in 4 (27%), palpitations in 2 (13%), and diarrhoea in 2 (13%) patients, but all of them continued therapy. We have found no case in thrombotic events. **Conclusions.** Anagrelide proved to be a safe and effective drug for pre-treated ET patients. The positive results described in several studies may well lead to the next use of anagrelide as first line treatment in patients with high risk ET.

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ANALYSIS OF 74 PATIENTS WITH PRIMARY MYELOFIBROSIS - IPSS DISCRIMINATE PATIENTS WITH A WORSE CLINICAL EVOLUTION

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Background. The use of risk scores for classification of patients with primary myelofibrosis (PM) allows a better evaluation and to test new therapeutic

options for the patients. **Aims.** evaluation of clinical and laboratory features of consecutive cases of PM in order to assign them to the risk stratification according to the International Prognostic Scoring System (IPSS). Validation of this score in our clinical setting. **Patients and Methods.** retrospective study of patients with PM, diagnosed between 1992 and 2011 at our Institution. Patients' diagnosis was revised according to 2008 WHO diagnostic criteria. At diagnosis we evaluated peripheral blood cell counts, circulating blasts, JAK-2 V617F mutation, splenomegaly and constitutional symptoms. Patients were stratified according to IPSS score. Overall survival (OS) was calculated from diagnosis until last follow-up or death and progression-free survival (PFS) from diagnosis until progression to acute myeloid leukemia, last evaluation or death. Informed consent was asked for participation in the study from patients (or family when patient was dead). **Results.** We examined 74 patients. Median age = 71. 5 years (31- 92). At diagnosis: Hemoglobin =5. 4-16. 9 g/dL (median = 12. 2), white blood cell count = 0. 9 - 47. 3 x 10^9/L (median 11. 4) and platelets =76-1. 545 x 10^9/L (median 456). Thirty-one patients (42%) presented splenomegaly, and cytogenetic abnormalities were found in 1/37 evaluated (partial deletion of chromosomes 13 and 15). JAK2/V617F mutation was present in 47% of the cases, and was related to a worse OS in 10 years (39% *vs* 77%), though not statistically significant (P=0,448). Patients were stratified according IPSS classification in low risk: 15 (26%), intermediate-1: 23 (32%), intermediate-2: 19 (26%) and high risk: 15 (31%). Treatment: 66 patients (89%) were treated with Hydroxiurea, changed to anagrelide due to toxicity (3 leg ulcers) or lack of response (3 patients). Six patients used thalidomide and prednisone, 3 as initial treatment. Three patients were exclusively treated with transfusion support and three patients received no treatment. Two patients were submitted to splenectomy and 3 cases to splenic irradiation. One patient was submitted to allogeneic bone marrow transplantation, with complete remission of the disease. Thrombotic events were observed in 16% of the patients. The presence of V617F JAK-2 mutation was associated with thrombosis (p=0,037). During follow-up 15 patients died: 9 due to infections, 3 after blast crisis and 3 not related to MF. Patients with IPSS intermediate-2 and high risk presented a worse OS in 10 years (low: 92%; interm-1: 67,5%; interm-2: 28%; high risk: 32%) (P= 0,02). **Conclusions.** this study confirms the importance of IPSS for risk factor stratification. A correlation was found between V617F JAK-2 mutation with thrombosis. Support treatment with hydroxiurea is able to control low risk patients in the cellular phase of the disease. Patients with intermediate and high-risk disease are candidates to other therapeutic approaches, as bone marrow transplantation or experimental drug therapies.

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ANAGRELIDE IN MONOTHERAPY OR COMBINED WITH HYDROXYUREA YIELDS A HIGH RATE OF COMPLETE RESPONSE IN ESSENTIAL THROMBOCYTHEMIA

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Background. Hydroxyurea (HU) and Anagrelide (Ana) are effective in reducing platelet counts in patients with Essential Thrombocythemia (ET). Nevertheless Ana can be used as second line treatment for ET patients resistant or intolerant to first line therapy, according to its registration to EMEA. **Aims.** We want to describe our experience with these drugs and our clinical findings in terms of hematological response. **Patients and Results.** We evaluated 113 ET patients treated with HU, Ana or a combination of these drugs. Twelve patients were treated with anagrelide (Ana); of these, 2 patients asked for Ana as first line therapy while 10 were resistant to first line treatment after a median of 43 months. After 21 months of therapy, median Hb, PLT and WBC values were respectively: 13. 6 g/dl, 449 x10^3/mm^3 and 8665/mm^3 (data calculated as the median of these parameters for each patient, based on determinations made every two months). Six patients (50%) achived a complete response (CR) with PLT normalization; doses ranged from 1 to 1. 5 mg daily. Five patients experienced partial response (PR), with PLT < 600 x10^3/mm^3 or a decrease >50% from baseline; only one was resistant. Mild side effects (transient headache and palpitation) were observed in 30% of them. No advers reaction led to therapy discontinuation. Ninety-three patients received HU as first line therapy: 23. 6% achieved CR, 55. 4% PR and 21% were resistant to HU. Hb, PLT and WBC median value were respectively 13. 5 g/dl, 406 x10^3/mm^3 and 6570/mm^3. We used also combined HU and Ana therapy in 8 patients resistant to multiple line treatment, included busulfan (2 patients). We used 1 g daily of HU plus escalating Ana doses (from 1 to 2 mg daily), on the basis of their individual hematological responses. After a median of 23 months Hb, PLT and WBC median value were respectively 13. 7 g/dl, 456 x10^3/mm^3 and 7355/mm^3. Five patients (62,5%) achieved CR and 3 PR (HU 1 g plus Ana 2 mg daily). None was resistant to association therapy. **Conclusions.** Although groups treated with Ana plus HU or Ana as monotherapy could represent a selection bias because they include patients resistant to previous treatment, the rate of CR is higher in these groups, compared to those on HU therapy. Despite resistance to previous treatment Ana seems effective, with only one patient being resistant to this treatment. Nevertheless, Ana plus HU is more useful in controlling WBC count compared to Ana monotherapy. Combined therapy or Ana monotherapy should be considered also in patients with PR to HU, to try to achieve CR and the best control of platelet lowering.

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SPLEEN IS THE HIDDEN HITCH OF ESSENTIAL THROMBOCYTHEMIA

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Background. Splenomegaly occurs in about 60% of Essential Thrombocythemia (ET) patients, and normalization in spleen size is one of the clinical-hematological criteria for assessment of response to treatment, according to the European Leukemia Net criteria, although it is referred only to craniocaudal diameter (CCd). **Aims.** This study aimed to investigate the most precise parameter between spleen volume (SV) and CCd in defining splenomegaly in ET patients and to predict the reciprocal impact of splenomegaly and clinical outcome. **Methods.** SV and CCd of 170 ET patients were measured by ultrasound at the time of diagnosis. The same operator performed all the measurements evaluating perimeter and longitudinal diameter while area and volume were calculated automatically. **Results.** As many as 69% of patients showed SV >250 mL (<250 is considered the normal value), while 48% of them had a CCd >11. 5 cm (<11 nv). Median SV and CCd were 360 mL and 11. 8 cm respectively. Although there was a correlation between SV and CCd (Pearson. 750), 25% of patients showed normal or reduced CCd and SV enlargement, at the same time. At follow up (median 56 months), they showed significant increases in SV (p<0. 001), median SV being 550 ml, with a 92% average increases. The correlation between SV and CCd (Pearson. 864) was stronger. When we correlated the difference in average SV with that CCd, we found a reduced correlation (Pearson. 24), because some patients (20%) showed SV enlargement with a simultaneous reduction in CCd. There is no statistically significant difference between hematological response and SV enlargement, although in no responder patients SV seemed to increase slight (p=0. 087). On the other hand, type of treatment influences SV independently to the response. IFN treated patients showed the highest increase (mean=670 mL; p=0. 001), followed by anagrelide (mean=639 mL; p=0. 017) and untreated (mean=481 mL; p=0. 023) patients. A no significant enlargement was observed in HU treated patients, p=0. 1. SV increased in both JAK2V617F and JAK2WT patients, p<0. 001 and 0. 002 respectively. We evaluated MF grading in 44 patients at follow up: in 34. 1% fibrosis worsened, in 36. 4% it was stable and in 29. 5% it improved. All patients had SV enlargement but the first group showed the highest increase. Patients in whom MF increased had the highest SV at baseline (p=0. 004). Patients with CR showed the lowest rate of MF worsening grade. In 55. 5% of anagrelide treated patients MF improved, against 33. 3% of those on HU and 26. 7% of those on IFN treatment. **Conclusions.** Spleen volume is the most discriminative and precise parameter to catch and monitor splenomegaly. Traditional ET treatments usually fail to reduce spleen size in patients, therefore volume normalization should be considered anecdotic. There is a difference in spleen response to different treatments and this finding should be better evaluated, in view of the newly available drugs that reduce spleen size. Further, highest SV at diagnosis should be considered prognostic of evolution tendency of the disease toward worsening of MF grade. This suggests the need for accurate monitoring in these patients.

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THE INCIDENCE OF THROMBOHEMORRHAGIC EVENTS IN ESSENTIAL THROMBOCYTEMIA. ROLE OF THE TREATMENT WITH ANAGRELIDE

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Background. Essential thrombocythemia (ET) is a myeloproliferative disorder characterized by persistent thrombocytosis. The recent discovery of JAK2-V617F mutation is a major break-through in understanding the molecular pathogenesis of the MPDs. Despite decades of clinical and laboratory research, relatively little has been accomplished concerning the pathogenesis as well as the identification of risk factors for thrombosis and bleeding in myeloproliferative disorders. Anagrelide is the latest addition to the therapeutic arsenal in platelet lowering therapy in myeloproliferative disorders especially in essential thrombocythemia (ET). Anagrelide selectively reduces the production of platelets by inhibiting megakaryopoiesis. Its efficacy is about a 70% in ET and the response

is rapid (in a few weeks). **Aims.** The incidence of thrombohaemorrhagic events at 45 patients with ET in a hemato-oncological center of Timisoara over a period of 3 years and to determine the efficacy and long-term safety of anagrelide. **Methods.** We included 45 patients with ET, 24 men and 21 women, with a median age of 39 years. Splenomegaly was present in (16,2%) patients and fibrosis in (22,3%) . The positive diagnosis is sustained by WHO criteria for ET:- sustained platelets count>450x10^9/l -bone marrow biopsy-demonstration of JAK2617V>F. 45% of patients received a prior treatment with one of two cytostatic agents and 55% received as a first line anagrelide. **Results.** The most important **t**hrombohemorrahagic events were: splenic thrombosis - 10,01%, cerebral thrombosis - 33,06%, pulmonary thrombosis - 5,21%, portal vein thrombosis - 2,32%, peripheral thrombosis - 9%, cutaneous bleeding - 6,10%, mucosal bleeding - 4%, upper gastrointestinal bleeding - 0,71% At 85 % of the patients treated with anagrelide platelet levels fall below <450x10^9/l. 25% developed toxic side effects as tachycardia. In the first two weeks of treatment 33% of the patients suffered from nausea and vomiting. **Conclusions.** Thrombosis in ET occurs in arterial, venous or microcirculatory locations. In general, arterial events predominate over venous events. Of importance, patients with ET have an unusually high rate of intra-abdominal (portal and hepatic) vein thrombosis and together account for a substantial proportion of identifiable causes of these potentially catastrophic events. Interestingly, young patients appear to be particularly vulnerable to this complication. There is increasing evidence to suggest an additional role by leucocytes that might partly explain the antithrombotic effects of myelosuppressive therapy. Complete remission (defined as a platelet count<400x10^9/l in symptomatic patients and 600x10^9/l in asymptomatic patients) was maintained in 75% of patients still treated with anagrelide. Long term efficacy was good, tolerance and safety were satisfactory and the cardiac toxicity was low. No thrombohemorrhagic events after introduction of Anagrelide in treatment of patients.

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IS JAK2 V617F GENE POINT MUTATION A RISK FACTOR FOR BLEEDING OR THROMBOSIS IN PATIENTS WITH MYELOPROLIFERATIVE DISEASE?

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JAK2 V617F gene point mutation in patients with myeloproliferative diseases (MPD) were firstly defined in 2005 and their prevalence by diseases is 90% for polycythemia vera (PV), 50-60% for essential trombocytosis (ET) and 50-60% for primary myelofibrosis (PMF). Currently, the disease course of the positive JAK2 V617F gene point mutation (JAK V617F$^+$) and especially the effect on bleeding/thrombotic complications still are not well-understood. In this study, the data of 94 patients with myeloproliferative disease (median age: 57 (20-80), PV: 39, ET: 49, PMF:(6) who were admitted to the hematology clinic of Meram Medical School, Konya University between 2000 and 2011 were evaluated retrospectively. The number of JAK V617F$^+$ cases was 40 (42. 5%), (median age: 61), the number of JAK 2 V617F$^-$ cases was 54 (57. 5%) (median age: 54). The ages of JAK V617F$^+$ cases were found to be significantly higher. When the distribution by the diseases was considered; it was found that the number of PV-JAK V617F$^+$ cases was 21 (54%), (median age: 66), the number of the negative cases was 18 (46%) (median age: 53). The median age of JAK V617F$^+$ cases was found to be significantly higher. The number of ET- JAK V617F$^+$ cases was 16 (33%), (median age: 60), the number of negative cases was 33 (67%) (median age: 51), and the age of the positive cases was again found to be significantly higher. But, There was no found significant difference between PMF- JAK2 V617F$^{+/-}$ in patients. When the bleeding/thrombotic event distribution was considered; there were no events in 38 (70%) of the JAK 2 V617F$^-$ cases, there was an event in 16 (30%) cases (n: 10 (18. 5%) bleeding, n: 6 (11%) thrombosis). There were no events in 16 (40%) of JAK V617F$^+$ cases and an event was seen in 24 (60%) (Bleeding: 17 (42. 5%), Thrombosis: 7 (17. 5%)). The event incidence in JAK V617F$^+$ cases was found significantly higher (60%). There were 21 PV- JAK V617F$^+$ cases (Bleeding: 11, Thrombosis: 5, total: 16 (71%)), 16 ET - JAK V617F$^+$ cases (Bleeding: 5, Thrombosis: 3, total: 8 (50%), 3 MPH- JAK V617F$^+$ cases (Bleeding: 1, Thrombosis: 0) total: 1 (33%). In cases with PV and ET, the incidence of the events including bleeding and thrombosis were significantly higher. Bleeding and thrombotic complications observed during the course of myeloproliferative diseases are among the most important morbidity and mortality causes. In the literature, there are studies reporting that the diagnosis of myeloproliferative diseases increases the thrombotic complication risk of JAK V617F$^+$, which has been started to be routinely used especially for PV. In this study, it was found that the ages of JAK V617F$^+$ patients were significantly high and there was an increased bleeding/thrombotic complication risk. In conclusion, in the light of the literature and our findings, it is possible to recommend a closer monitoring for bleeding/thrombotic complications of elderly MPD- JAK V617F$^+$

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CLINICAL OUTCOME OF 15 PREGNANCIES IN WOMEN WITH CHRONIC MYELOID LEUKEMIA AND CHRONIC MYELOPROLIFERATIVE DISEASES PH NEGATIVE - THE EXPERIENCE OF FUNDENI CLINICAL INSTITUTE, BUCHAREST, ROMANIA

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The incidence of myeloproliferative diseases increases during the reproductive ages. Pregnancy in female patients with childbearing potential and chronic myeloproliferative disorders can occur accidentally or as their own wish. During the last 10 years in Fundeni Clinic of Hematology were registred 15 female patients with chronic myeloproliferative diseases (3 chronic myeloid leukemia, in chronic phase, treated with Imatinib, 6 essential thrombocythemia and 2 cases of polycythemia vera) who became pregnant . 8 complete healthy babies were born without delivery or post-partum complications. 1 patient with ET had 2 consecutive pregnancies. 3 patients underwent abortions (1 because of ectopic pregnancy, 2 on demand). A patient diagnosed with PV-ET jak2 positive and factor V Leiden had fetal loss because of recurrent placentar thrombosis and fetal involution. All the patients with ET and PV received low dose of Aspirin throughout pregnancy and prophylactic low molecular weight heparin in the 3rd trimester and 6 weeks postpartum. In high risk (5 cases)ET patients alpha interferon was the preferred cytoreductive therapy. There were no maternal complications after delivery and no fatal event. The hematologic response and an appropriate monitoring is the key for an event free pregnancy and an optimal delivery.

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HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISEASES

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Myeloproliferative diseases (MPD) belong to the group of clonic malignant diseases of parent cell hematopoiesis, characterized by abnormal increase of one or several blood lines with normal or nearly normal maturing of those cells, both in bone marrow and in extramedullary hemotopoietic organs. According to the literature data, in patients with CML a tendency to bruises and bleeding was described in about 35% of patients. In patients with PV, hemorrhagic changes on the skin and visible mucous membranes are common as the disease progresses. Bleeding occurs in 15-18% of patients with IMF. Even 60% of patients with ET showed tendency to hemorrhage, which is a major cause of morbidity and mortality. The aim of the paper is to demonstrate the extent to which the hemorrhagic complications occur in patients with certain forms of chronic myeloproliferative diseases. The investigation included 219 subjects of both sexes, between 17 and 83 years of age with the diagnosis of MPD. The patients were divided into five groups: A. Chronic myeloid leukemia (CML)-group; B. Polycythaemia vera (PV)-group; C. Idiopathic myelofibrosis (IMF)-group; D. Essential thrombocythaemia (ET)-group; E. Myeloproliferative disease that cannot be classified (MPS)-group. During the investigations the methods of clinical examination were used, as well as laboratory examination methods (biohumoral parameters and coagulation status). In our investigation, hemorrhagic changes in terms of bruising, ecchymoses of the skin, epistaxes, gingival bleeding were described in 16% of patients with MPD. The presence of ulcer disease with hemorrhagic complications was observed in slightly less than a fifth of patients with MPD (19. 18%). In groups with PV, ET and MPS, between 30 and 40% of patients were with ulcer disease, which is statistically significantly higher percentage compared to the patients with CML. Also, a small percentage of our patients with ET have a hemorrhagic syndrome in terms of changes on the skin, but those patients have a significantly higher incidence of ulcer disease (35%), and thus the complications in terms of gastrointestinal bleeding in those patients were significantly higher. When the patients with PV and IMF are in question, there is an agreement with literature data in terms of hemorrhagic syndrome occurrence. Bleeding in MPD is caused by thrombocytopenia, qualitative platelet disorders, acquired factor V deficiency and vWF, disseminated intravascular coagulation. Thrombocytopenia occurs due to ineffective megakaryocytopoiesis, retention of platelets in the enlarged spleen and platelet decomposition due to DIC. The disorder of platelet function results in prolonged bleeding time, low platelet aggregation particularly on adrenalin and abnormal content of beta- thromboglobulin, 5-hydroxytryptamine and platelet factor 4. We have proved that the prevalence and severity of hemorrhagic complications are in statistical dependence on the type of MPD. Hem-

orrhagic complications often accompany chronic myeloproliferative diseases. Hemorrhagic syndrome in the form of changes on the skin and visible mucous membranes is more common in patients with IMF and CML during blast crisis phase, while the severe gastrointestinal bleeding was statistically and more significantly present in patients with ET and PV.

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PREVALENCE OF JAK2V617F MUTATION AND ITS CLINICAL CORRELATION IN THAIS WITH MYELOPROLIFERATIVE NEOPLASM

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Background and Aims. Myeloproliferative neoplasm(MPN) is a group of chronic myeloid cancers. According to the WHO 2008 recommendation, JAK2V617F mutation is a major diagnostic criterion for these diseases, particularly theP-hiladelphia chromosome-negative MPN. The prevalence of such mutation, however, has been reported differently in numbers based on the study population; rarely Thais. This study aims to explore the prevalence of the JAK2V617F mutation in Thai MPN patients and examine its correlation with clinical features. **Methods.** 103 Thai MPN patients, diagnosed according to the WHO 2008 criteria, were recruited and blood samples were taken for DNA isolation. JAK2V617F detection was explored using two PCR based techniques; AS-PCR and RFLP. Clinical data assessed at the diagnosed date were matched to the JAK2 mutation outcome. **Results.** The overall prevalence of JAK2V617F in MPN was 64. 1% (66/103); 59. 2% (29/49) in ET, 80. 6% (25/31) in PV, 70. 0% (7/10) in PMF, and 83. 3% (5/6) in MPN nos. None of the CML patients had the mutation (0/7). The mutant group had significantly higher red blood cells (P = 0. 030) and less bleeding history (P = 0. 011) than the wild-type (WT). We found no statistical difference of any clinical features between those two groups in ET, albeit some haematological parameters, white blood cell and platelet count, of the mutant were slightly higher than the WT without significance (P= 0. 098**,** and 0. 850). Haemoglobin, haematocrit and MCV of the mutant PV were lower than the WT while the red blood cells and platelets were higher. **Summary and Conclusions.** JAK2V617F does exist in Thai MPN patients. Its prevalence is close to other populations of different ethnicity. The mutant group displayed higher haematological parameters referring to contribution of the mutation to the disease. Nevertheless, other factors including vascular evidences and intracellular signal transduction molecules could altogether participate to cause the phenomenon.

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STUDY OF MULTIPLE CANCER WITH THREE OR MORE CANCERS INCLUDING HEMATOPOIETIC MALIGNANCY -EXPERIENCE OF 17 CASES IN OUR DEPARTMENT

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Introduction. The recent advances in examination techniques and medical knowledge has enabled the early detection of cancer, and the indication for surgical treatment has been expanded by improved radical surgical techniques, changing cancer from a fatal disease to a treatable disease. In some cases, however, it has become difficult to determine the therapeutic policy when a solid cancer is detected during the treatment process. Herein, we report 27 cases with three or more cancers including hematopoietic organ tumor and solid cancer. **Methods.** Among 145 patients who were diagnosed as having hematopoietic malignancy and multiple cancer at our hospital during the period from 1988 to 2009, 27 patients with three or more cancers were selected as the subjects. **Results.** Among 145 patients with multiple cancer, including hematopoietic malignancy, 27 patients (18. 6%) had three or more cancers. The subjects (22 males and 5 females) included 25 patients with triple cancer and 2 patients with quadruple cancer. The median age at which triple cancer was diagnosed was 68 years old (42 - 86 years old), and the triple cancer developed as synchronous cancer in 9 patients and as metachronous cancer in 18 patients. The median age for patients with synchronous cancer was 67 years old, while that for patients with metachronous cancer was 68 years old. The 83 diagnoses among the 27 patients included esophageal cancer in 2 patients, stomach cancer in 11, bowel cancer in 8, hepatic cancer in 5, pancreatic cancer in 1, cholangiocellular cancer in 1, lung cancer in 7, renal cancer in 6, urinary bladder cancer in 2, prostate cancer in 5, thyroid cancer in 2, meningioma in 1, and hematopoietic malignancy in 31. Regarding the 18 patients with metachronous cancer, it was found that 9 patients were diagnosed as having developed 3 types of malignant tumor independently, that 7 patients developed the 3rd cancer after being diagnosed with double cancer, and that 2 patients simultaneously developed 2 types of malignant tumor after undergoing treatment for the 1st cancer. **Discussion.** Campbell et al. reported that the incidence of a 3rd cancer in patients who had developed a 2nd cancer would be 8. 94%, which is more than double the incidence of the 2nd cancer (3. 97%). It has been suggested that multiple cancers tend to develop in the organs that are predisposed for single cancer. In Japan, the incidence of multiple cancer, including stomach cancer and cancer of other digestive organs, is high, while the incidence of multiple cancer including skin cancer is high in the US and European countries. In the future, it is expected that development of multiple cancer will increase along with the advances of diagnostic techniques and improvement of therapeutic results. In the diagnosis and treatment of cancer patients, it will be important to always consider the possibility of multiple cancer, and to perform follow-up observation for patients who undergo radiotherapy and administration of anticancer drugs, paying sufficient attention to the development of metachronous cancers.

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CORRELATION BETWEEN HEMATOCRIT AND THROMBOTIC RISK IN OUR POLYCYTHEMIA VERA PATIENTS

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Background. Polycythemia Vera (PV) is a disorder of haematopoiesis included within the myeloproliferative neoplasm in the forthcoming revised World Health Organization (WHO) classification. The discover of acquired recurrent molecular abnormalities in JAK2 has improved his diagnostic approach. The main goal of therapy is therefore to prevent thrombohemorrhagic complications and this is effectively and safely accomplished by the use of hydroxyurea, low-dose aspirin and phlebotomy to keep Hematocrit less than 0. 45L/L as recommended by the recently published ELN criteria. Other studies, however, have reported that it does not seem necessary to maintain patients at all time below such threshold. **Aims.** We would like to try to establish the association between moderately high hematocrit values (>0. 45L/L < 0. 50L/L) and thrombotic risk. **Methods.** We reviewed the medical charts of all patients with PV in our institution. In every case, the diagnosis of PV was reassessed using the updated criteria of the WHO and our hematocrit target was defined as less than 0. 45L/L according to the recently published ELN criteria. In all cases the occurrence of thrombosis and bleeding were registered. **Results.** In total 50 patients were included in the study. Their main clinical and haematological data at diagnosis are listed as below: Age 65y (mean value), Sex, male/female 25/25, cardiovascular risk factors n°25 (50%), PV-related symptoms n°20 (40%), Palpable splenomegaly n° 10 (20%), Leukocytes >10x109/L n° 25 (50%), Platelets > 500 x 109/L n° 25 (50%), JAK2 mutation n° 50 (100%). All the patients received antiplatelet therapy and median interval between diagnosis and HU start was 0. 3 years and the reasons for initiating HU were established according to SIE therapy guidelines. In the majority of patients the initial dose was of 1000 mg/day and it was subsequently adjusted to keep the hematocrit below 0. 45L/L. However in some cases (about 50%) during the follow-up we had a moderately high hematocrit values (>0. 45L/L < 0. 50L/L). Thrombosis and bleeding were not observed in all the cases and with the same rate over the periods in which patients remained with hematocrit less than 0. 45L/L versus the periods in which the same patients remained with hematocrit major than 0. 45L/L but less than 0. 50L/L. **Summary and Conclusions.** Thrombosis is the main cause of morbidity and mortality in Polycythemia vera and its pathophysiology is complex. Traditionally, increased hematocrit has been claimed as the main player, but the dynamic interactions between platelets, leukocytes, and the endothelium do probably represent a fundamental interplay in generating a thrombophilic state. Taken into consideration our results it would be advisable targeting the hematocrit at 0. 45L/L but it does not seem necessary maintain patients at all times below such threshold. Probably, the identification of plasma markers translating the haemostatic imbalance in patients with PV would be extremely helpful in order to better define the subgroup of patients with a significant clinical risk of thrombosis.

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IMPACT OF V617F MUTATION IN THE MANAGEMENT OF THROMBOTIC RISK IN PATIENTS WITH ESSENTIAL THROMBOCYTEMIA

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Background and Aims. The V617F JAK-2 mutation, which occurs in 40-50% of patients with essential thrombocythemia, seems to be associated with an

increased thrombotic risk, although the therapeutic criteria have not been modified for this group. We have studied 66 patients with essential thrombocythemia (ET) and V617F mutation with the aim to rely clinical and laboratory characteristics and treatments received with thrombotic risk. **Patients and Methods.** We reviewed retrospectively the medical records of 66 patients diagnosed of essential thrombocythemia according to WHO criteria during the period of time between 1988th to April 2011. All of them were also positive for the V617F mutation PCR performed using allele-specific real-time. The median age group was 62. 5 years; 37,87% were male and 62,12% were women. 25% of patients had splenomegaly at diagnosis and a median of 805x10e9 platelets / L. According to international criteria for the treatment of ET, 27. 3% of patients were ASA antiplatelet therapy alone, 54. 5% received treatment with hydroxyurea + /-ASA, 10. 6% received treatment with anagrelide + /-AAS, the 3% was in the combined treatment with anagrelide and hydroxyurea plus 4. 5% received no treatment. The10,6% were homozygous for the mutation V617F JAK-2 and 89,4%were heterozygous. 66. 6% of patients had cardiovacular risk factors (diabetes, hypertension, hyperlipidemia) Using SPSS statistical application we analyzed the influence of ag, platelet count, presence of splenomegaly, treatments received and allelic burden on thrombotic risk. **Results.** 34. 8% of patients had a thrombotic event. We found a significant difference in platelet count in patients with thrombosis (median949x10e9 / L) versus those without (median 759x10e9 / L), with p= 0. 03%. The presence of cardiovascular risk factors in our group also conditioned increased risk of thrombosis (91. 3% Yes vs. No8. 7%, p = 0. 002%). The other factors analyzed showed no impact on the thrombotic risk. The group's overall survival was 91. 7%. **Conclusions.** The results in our group confirm the high risk of thrombosis in patients with essential thrombocythemia and JAK-2 V617Fmutation, with a thrombotic event producing in at least one of every 3 patients. The median platelet count in this group, less that recommended for initiating treatment in most of the accepted protocols for TE, and cardiovascular risk factors will be the determining of the therapeutic approach in V617F ET as a different clinic entity.

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CONTRIBUTION OF ARRAY COMPARATIVE GENOMIC HYBRIDIZATION TO PROGNOSTIC STRATIFICATION IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Diffuse large B-cell lymphoma (DLBCL), a heterogeneous clinicopathologic entity, accounts for up to 40% of non-Hodgkin's lymphomas. Gene expression profiling confirmed the presence of three molecular prognostic subgroups - germinal center B-cell-like (GCB), activated B-cell-like (ABC) and primary mediastinal B-cell lymphoma (PMBL). Nevertheless, there is a need to identify genes with a critical function in DLBCL to stratify patients into prognostic subgroups without expression profiling. **Aims.** To study copy number alterations (CNAs) in DLBCL patients and to correlate them with cytogenetic and other molecular cytogenetic results to define the optimal approach for prognostic stratification. **Methods.** A single-center study comprising 51 DLBCL patients (24 males; median age at diagnosis 49 years). Thirty-two patients were histopathologically diagnosed as DLBCL, 18 patients as PMBL and one patient as gray zone lymphoma. ArrayCGH with BAC microarrays was used in 26 patients (Bluegnome, Cambridge, UK; K3 Leiden, Netherlands) and oligonucleotide microarrays 4x44K (Bluegnome and Agilent, Santa Clara, USA) were applied in 25 patients. Fifty patients were examined by conventional cytogenetics; FISH with different probes was applied in all patients. **Results.** Stratification criteria published by Staudt and Dave (2005) were used. ArrayCGH revealed CNAs in 28 (55%) patients. The most frequent losses were detected on chromosomes 1q, 4q, 6q, 13 and 17p; the most frequent gains on chromosomes 7, 8q, 9p and 12. Based on the results, all 28 patients were stratified into molecular subgroups. Using conventional cytogenetics, chromosomal changes were detected in 26 (51%) patients. The most frequent balanced chromosomal change was t(14;18) confirmed in 6 patients. *BCL2* duplication was confirmed in 5 patients. FISH analysis of 45 patients with locus-specific probes LSI IGH/MYC,CEP8 and/or LSI MYC DC (Abbott Molecular, Illinois, USA) revealed low frequency of translocations (1 patient) in the cohort but frequent presence of *CMYC* gene duplication (11/45 patients). The overall survival of patients with *CMYC* aberrations was shorter compared with those without them but a larger cohort is needed. The gain of the *JAK2* gene was studied in 25 patients with locus-specific probes ON JAK2 (9p24) Break (Kreatech, Amsterdam, Netherlands). Duplication and amplification were confirmed in 5 and 7 PMBL patients, respectively. Conventional cytogenetics, FISH and arrayCGH enabled detection of 28 (55%) patients with a complex karyotype. With all results obtained from cytogenetic and molecular cytogenetic methods, a total of 45 (88%) patients could be stratified. There were 11, 14 and 20 patients in the ABC, GCB and PMBL subgroups, respectively. **Conclusions.** Prognostic stratification based on arrayCGH was performed in 55% of patients. With a combination of all cytogenetic and molecular cytogenetic methods, a total of 45 (88%) patients were stratified. The results confirmed the benefit of arrayCGH for detecting CNAs in DLBCL for prognostic stratification but also to the necessity to supplement the result by FISH and cytogenetics to detect balanced changes of prognostic and stratification importance. The detection of all as well as cryptic copy number changes could possibly reveal differences in the oncogenic mechanisms in DLBCL. Supported by MZČR IGA NT 11103.

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HNRNP B1 INHIBITS DNA DOUBLE-STRAND BREAK REPAIR BY INTERACTION WITH DNA-PK COMPLEX IN ADULT T-CELL LEUKEMIA

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Adult T-cell leukemia (ATL), an aggressive lymphoid malignancy caused by HTLV-1, is associated with a large variety of genetic alterations. We previously reported that heterogeneous nuclear ribonucleoprotein (hnRNP) B1 is bound to the DNA-dependent protein kinase (DNA-PK) complex, inhibits DNA repair in lung cancer cell lines; we therefore investigated the expression of hnRNP B1 and its effects on DNA repair activity in ATL cells. hnRNP B1 was overexpressed in 35 of 48 (73%) lymphoma tissues from ATL patients and the level of expression was correlated with serum soluble interleukin-2 (IL-2) receptor level. An increase in hnRNP B1 protein level occurred in the CD4-enriched cell fraction in asymptomatic HTLV-1 carriers and was associated with stage of the disease. The protein level of hnRNP B1 was associated with IL-2 stimulation in ATL cell lines, as well as normal CD4-positive T cells. Although constitutive expression of *hnRNP B1* mRNA without IL-2 stimulation was observed in ATL cell lines, transcriptional activation of *hnRNP B1* mRNA was induced by IL-2 stimulation in normal CD4-positive T cells. These results indicate that hnRNP B1 protein is induced by growth factor IL-2 in normal CD4 positive cells, mediated by transcriptional mechanisms, but in cancer cells *hnRNP B1* gene activation is IL-2 independent and constitutive. Functional analysis revealed that hnRNP B1 interacted with the DNA-PK complex and inhibited kinase activity of DNA-PK as well as double-strand DNA break repair in ATL cell lines. Our results suggest that overexpression of hnRNP B1 may play an important role in accumulation of genetic abnormality and disease progression of ATL mediated through inhibition of DNA-PK activity.

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CD4+ T-CELL POPULATIONS IN DIFFERENT MICROANATOMICAL LYMPH NODE COMPARTMENTS OF FOLLICULAR LYMPHOMA

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Background. Subpopulations of CD4+ T-cells, including T-regulatory cells (Treg) in follicular lymphoma (FL) microenvironment play important role in control of malignant cell growth either directly or via influence on effector cell populations. **Aims.** To characterize CD4+ cells, its regulatory subpopulations (FOX-P3, PD-1) as well as CD8+ cells and to ascertain their relationships in different microanatomical lymph node compartments (follicular, interfollicular, when present - parafollicular and diffuse) in follicular lymphoma. **Methods.** We have studied 73 patients with follicular lymphoma. In all cases the diagnosis was confirmed with immunohistochemistry. Patients were investigated according to standard clinical and laboratory tests. Special quantitative study (percentage of positive cells within each compartment) of T-cell subpopulations (CD8, CD4, FOX-P3, PD-1) was done by immunohistochemistry on paraffin sections of FL biopsy specimens. **Results.** We have studied 73 FL patients (male - 26, female - 47) aged from 25 to 91 years (median - 58). Cytological grade II predominated (55 pts), grade I - 5, grade IIIa - 13. Stages according to Ann-Arbor classification were as follows: I -4 pts, II - 11, III - 10, IV - 46. Distribution according to FLIPI: good (score 0-1) - 15 pts, intermediate (score 2) - 13, poor prognosis (score 3-5) - 30; according to FLIPI2 - intermediate (1-2) - 15, high risk - 16. Distribution of different T-cell populations is given in Table 1. We did not found any negative correlations between numbers of cytotoxic (CD8) and regulatory/suppressor (FOX-P3, PD-1) T-cell populations in each microanatomical compartment. Moreover we noticed some strong positive correlations (p <0. 01 - <0,05) for some subpopulations studied. Number of CD8+ cells correlat-

ed with the total number of CD4+ lymphocytes and PD-1+ cells both for interfollicular and for intrafollicular populations, the same was true in cases with diffuse pattern of infiltration. This cytotoxic cell population (CD8) correlated with FOX-P3-cells only within follicules. CD4+ cells correlated with PD-1+ cells only in follicules and in diffuse areas of growth. Correlation between the number of regulatory cells (FOX-P3 and PD-1) was noted both within follicules and in interfollicular areas. No correlations noted for cells in parafollicular compartment may be explained by low number of cases in which this area may be clearly identified. **Conclusions.** Our data indicate that there are different numbers of CD4+, CD8+ and regulatory cell populations in different lymph node microanatomical compartments in FL. Elevated number of regulatory cells did not lead to reduction of cytotoxic cell population.

Table 1.

Subpopulation	Localization	Number cases	Range (%%)	M±m (%%)	Median
FOX-P3	interfollicular	47	1-30	12,4±1,2	10
	intrafollicular	62	1-30	10,5±0,9	10
	parafollicular	10	1-20	15,4±2,4	20
	diffuze	35	1-30	10,4±1,1	10
PD-1	interfollicular	50	3-80	19,6±2,1	15
	intrafollicular	63	2-60	22,7±1,9	20
	parafollicular	10	1-80	32,0±7,3	30
	diffuze	34	2-40	17,5±1,7	15
CD4	interfollicular	48	1-70	39,0±2,1	40
	intrafollicular	63	7-50	21,5±1,3	20
	parafollicular	5	8-80	32,6±12,6	30
	diffuze	33	2-50	24,6±2,5	20
CD8	interfollicular	49	2-30	18,8±1,0	20
	intrafollicular	62	3-30	9,4±0,6	10
	parafollicular	5	10-50	19,0±7,8	10
	diffuze	32	2-70	14,1±2,1	10

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BILATERAL BONE MARROW EXAMINATION FOR NON-HODGKIN'S LYMPHOMA STAGING: DIAGNOSTIC UTILITY IN THE MULTIPARAMETRIC FLOW CYTOMETRY ERA

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Background. Since the early 1970s, histopathological examination of bone marrow biopsy (BMB) is essential in the staging of non-Hodgkin's lymphomas (NHL). Bilateral BMB (BBMB) has been recommended, since several studies suggest it gives more accurate information than unilateral bone marrow biopsy (UBMB). However, the clinical value of this procedure has not been clearly established when other more sensitive techniques are used. We have retrospectively analyzed a series of 105 NHL patients with BBMB histological examination, in which four-color flow cytometry (FC) analysis was performed simultaneously. **Aim.** To evaluate BBMB's value when bone marrow FC analysis is used simultaneously in the routine staging of NHL. **Methods.** We analyzed 105 specimens from NHL patients, all of them with BBMB histological examination and four-color FC analysis on the BM aspirate performed simultaneously to the first BMB. When present, BM involvement was histologically classified as diffuse, nodular, interstitial and nodule-interstitial. Erythrocyte-lysed whole BM samples were stained using selected panels of monoclonal antibodies, using four-color direct immunofluorescence technique and according to previously well described methods, aimed to identify and characterize B and/or T neoplastic cells in BM. **Results.** Histological examination showed concordance in both BMB in 91% of cases (96/105), being both negative (BMB-/BMB-) in 73% (77/105) and positive (BMB+/BMB+) in 18% of cases (19/105). Histological discrepancies were seen in 9 cases, showing infiltration only in one of the BMBs (BMB-/BMB+). Therefore, performing a BBMB was determinant for a correct staging in 9% of cases (9/105 BMB-/BMB+). FC was positive in 6 of the 9 discrepant cases (BM-/BM+/FC+). Thus, the use of FC on the first BM aspirate would reduce the impact of performing a second BMB, since only 3% (3/105) of cases would have been considered negative when a second BMB have revealed BM involvement. Although FC was negative and concordant with histology in most BMB-/BMB- cases (87%; 67/77), interestingly in 10/77 cases (13%) showed a clonal population, revealing bone marrow involvement. As expected, cases with histological BM infiltration had higher levels of infiltration by FC than cases in which infiltration was only detected by FC, showing that FC has higher sensitivity in samples with low levels of infiltration. Taking into account the global series, the simultaneous use of FC and UBMB rises the detection of infiltration from 18% (19/105 cases BMB+/BMB+) with only one BMB, to 33% (35/105 cases: 19 cases BMB+/BMB+, 6 cases BMB-/BMB+/FC+ and 10 cases BMB-/BMB-/FC+), with a total improvement of 46% considering only cases with infiltration (16/35). In this context, a BBMB increased the detection of infiltration only by 3% [only 3 cases were negative in the first BMB with FC negative and showed infiltration in the second BMB (BMB-/BMB+/FC-)]. We have to consider also that a BBMB is an invasive procedure that implies costs, time and materials and supposes an important discomfort for the patient. **Conclusion.** According with our results we can conclude that the simultaneous use of UBMB and FC improves the detection of BM involvement and the staging procedure of NHL's, making a second BMB unnecessary.

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LYMPHOPROLIFERATIVE DISORDERS ASSOCIATED WITH THE SYNDROME OF ACQUIRED DEFICIENCY OF C1 INHIBIBITOR AND ANGIOEDEMA. RATE OF ASSOCIATION AND RESPONSE TO TREATMENT IN 48 PATIENTS

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Background. Expansion of B lymphocyte clones against C1-INH, ranging from monoclonal gammopathies of uncertain significance (MGUS) to non Hodgkin's lymphoma (NHL), seems to underlay the pathogenesis of angioedema due to acquired C1-INH deficiency (AEE), either associated with auto antibodies or with lymphoprolipherative diseases. **Aims.** Here we report on the long term follow up of 48 patients with AAE with specific focus on the fate of lymphoproliferative disease. Patients were followed for a median of 6 years (range 1-25). **Results.** Auto antibodies to C1-INH were positive in 35 of 48 patients (13 IgG, 13 IgM, 5 IgA, 2 IgG-IgM, and 2 IgA-IgM). Nineteen patients (40%) fulfilled diagnostic criteria for MGUS. MGUS and auto antibodies to C1-INH coexisted in 13 patients all, but one, sharing the same heavy chain isotype. In 7, binding of the M component to purified C1-INH could be demonstrated. In 12 patients (25%) NHL was diagnosed. Based on WHO classification 10 patients had indolent lymphoma, 1 high grade malignant lymphoma, large B cell, and one had a transformed high grade malignant lymphoma large B cell. Ten of the 12 patients with NHL received conventional chemotherapy. **Conclusions.** Various degrees of recovery of biochemical and clinical features of AEE were observed in all patients treated with chemotherapy. Our data support the existence of a tight association between B cell prolypheration and AAE pathogenesis.

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MEASURING OF TOTAL CELL DIVISION (THYMIDINE-KINASE) ACTIVITY WITH NOVEL SENSITIVE NON-RADIOMETRIC METHOD IN NHL AND CLL: STRONG CORRELATION WITH DISEASE STAGE AND ACTIVITY

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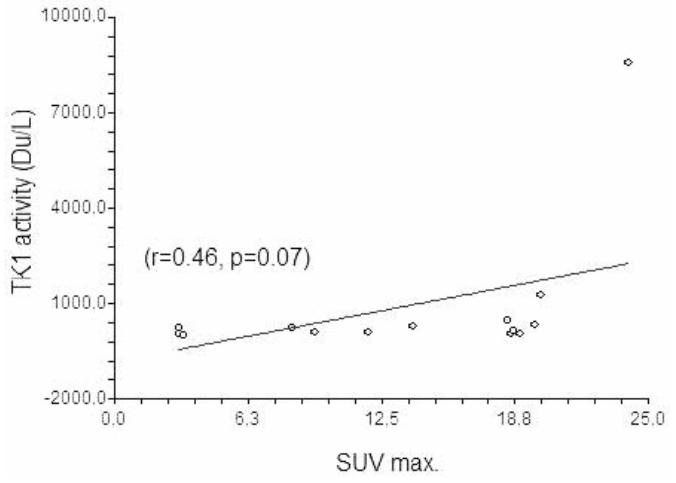
Background. Thymidine-kinase-1 (TK1) is a key enzyme expressed in the G1-S cell division stage to supply thymidine triphosphate necessary for cell division. Recent data show that pretreatment TK1 levels are predictive for survival of patients with non- Hodgkin´s lymphoma (NHL) or chronic lymphocytic leukemia (CLL). However, until now results are obtained with radioactive assays (TK-REA) with lower sensitivity and narrow detection range. **Aims.** To analyze the clinical-laboratory correlations of pretreatment TK1 levels analyzed with a novel non-radioactive high-sensitive method in unselected cohort of newly diagnosed patients with NHL and CLL and Hodgkin´s lymphoma (HL). **Methods.** Samples were obtained at time of diagnosis in 80 patients: 73 of them were diagnosed as lymphoma (diffuse large B-cell n=24, follicular n=3, marginal zone n=3, T-cell n=3 and Hodgkin lymphoma n=3, respectively) and 35 as CLL. In seven cases reactive (benign) lymphadenopathy was diagnosed. All samples were analyzed both with conventional TK-REA method and with a novel technique based on thymidine incorporation mediated by serum TK1 (DiviTum from Biovica Int AB, Uppsala, Sweden). Using novel method was mean TK1 value 590. 6±1260. 7 Du/L, median TK1 value was 195. 3 Du/L, TK-REA method assessed mean TK1 value 20. 4±22. 2 U/L (median 11. 8 U/L), both methods

showed strong correlation (r=0. 90, p<0. 001). We further analyzed patients with malignant lymphomas: median age was 64 years (range 22-89) with male predominance (M:F = 47:26), most of the patients (67%) had limited-stage disease (Ann Arbor stage I/II or Binet A/B), systemic symptoms were present in 43% and bulk >7cm in 30% of them. Beta-2-microglobulin (B2M) level was elevated >3mg/L in 21% and LDH in 32% of the cases. **Results.** Patients with reactive lymphadenopathy had significantly lower mean TK1 levels (163. 0±182 Du/L), compared to those with NHL, HL and CLL (631. 6±1312. 1 Du/L, p=0. 006). Mean TK1 level was significantly higher in patients with advanced disease stage (366. 8 vs 971. 0, p=0. 002), bulky disease (434. 4 vs 1036. 1, p=0. 006), systemic symptoms (335. 4 vs 979. 9, p<0. 001). Serum TK1 level was more than 3-fold higher in patients with high grade lymphoma than in low grade subtypes (317. 6 vs 1015. 0, p=0. 06) and 7-fold higher TK1 in patients with elevated LDH (209. 8 vs 1477. 7, p<0. 001). TK1 showed close correlation with LDH level (r=0. 79, p<0. 001). Trend was observed in correlation between maximum FDG avidity (SUV max.) on PET scan and TK1 level (r=0. 46, p=0. 07). On the other hand we found no correlation between TK1 and sex, age, B2M level and Ki-67 proliferating index. We found higher TK1 in CLL patients with unmutated IgVH status compared to those with mutated IgVH (607. 0 vs 166. 6, p=0. 002). **Conclusions.** The TK1 activity in specimens covers an extreme span - novel analytic method extends the detection range. It means that we are able to quantify higher levels than the conventional TK-REA method at time of diagnosis. We found a strong correlation between pretreatment TK1 levels and lymphoma stage and its aggressiveness.

Figure 1.



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E3330 ENHANCED ANTITUMOR EFFECT OF ADRINMYCIN ON HUMAN EXTRANODAL NASAL-TYPE NK/T CELL LYMPHOMA XENOGRAFTED-NUDE MICE

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Abstract. Extranodal Nasal-Type NK/T Cell Lymphoma is a distinct clinicopathological entity of non-Hodgkin's lymphoma, which is characterized by a highly aggressive clinical process and poor prognosis especially when disseminated or recurrent after radiotherapy. Human purinic/apyrimidinic endonuclease/redox factor-1 (hAPE/Ref-1) is a multifunctional protein involved in the repair of DNA damaged by oxidative. Because of its involvement in DNA repair and apoptosis-related signaling mechanisms, APE/Ref-1 is also being discussed as a novel target for tumor-therapeutic approaches. In studies, high level of APE1 expression is associated with an resistance to chemotherapeutic agents and adverse prognosis. And recent studies show that E3330, a small molecule inhibitor of the Ape-1/Ref-1 redox domain, induce inhibition of growth in cancer cell in vitro by specific inhibiting the function of APE/REF-1. E3330 blocks the in vitro growth of pancreatic cancer-associated endothelial cells and of the NK/T-cell lineage in our sdudy already and provides a potential therapeutic strategy in tumor . Thus far, no study has reported the experimental research on E3330 combined with chemotherapy in treating NK/T cell lymphoma. Therefore, we try to establish xenografted-nude mice model of human extranodal nasal type NK/T cell lymphoma and to investigate the effect of E3330 combined with adrinmycin on the human EN-NK/T-L xenografted-nude mice and its mechanism. **Methods.** EN-NK/T-L Xenografted-nude mice model was established by intraperitoneal injection of NK/T cell lymohoma cells SNK-6 . IC50 of E3330 for SNK-6 was calculated according to the result of MTT assay. Xenografted- nude mice were divided into four groups: Control group were given equal volume sodium□po□d1-7□ E3330 group were given E3330, 6mg/kg, po, d1-7□ADM group were given ADM, 0. 5mg/kg, ip,d1,4,7□E3330 + ADM group were given both E3330 and ADM, E3330, 6mg/kg, po, d1-7, ADM 0. 5mg/kg, ip, D1,4,7. The tumor weight and the survival rate of tumor-bearing mice were assayed, the apoptosis cells were determined by TdT-mediated Dutp Nick-end-Labeling(Tunel). Immunohistochemistry were used to study the expression of ki-67. Immunofluorescence were used to study the expression of APE/REF-1. **Results.** Nude mice model of human extranodal nasal type NK/T cell lymphoma was established successfully. The results showed that the expression level of APE/REF-1 was higher in tumor tissues compared with that in normal tissues (Fig 1 A 1B). The transplanted tumor volumes of mice were significantly smaller in E3330 + ADM group than those in other three groups(p<0. 05)(Table 1). The expression of ki-67 in E3330 + ADM group was lower than other groups(p<0. 05) (Fig 2 A 2B 2C 2D) and the apoptosis cells were increased in the same group(Table 2). **Conclusions.** Our study showed that E3330 + adrinmycin can significantly inhibit the growth of transplanted tumor of extranodal nasal type NK/T cell lymphoma Xenografted-nude mice .

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THE ASSOCIATION OF FOLATE METABOLISM GENES POLYMORPHISMS WITH RISK OF AGGRESSIVE NON-HODGKIN'S MALIGNANT LYMPHOMA

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Background. Non-Hodgkin›s lymphomas (NHLs) are a diverse group of malignant neoplasms, that affect lymphoid tissue. The major NHL subtypes is the aggressive Non-Hodgkin›s lymphomas, which most commonly diagnosed at advanced stages (III и IV). This fact implies that treatment efficiency will be low and disease prognosis will be poor. It is important to research new factors of the etiology of this disease, such as multiple single nucleotide polymorphisms (SNPs) in the genes implicated in various molecular pathways that lead to NHL. Among these genes are folate-metabolizing genes. **Aims.** The Aim of this study was to investigate the role of the C677T and A1298C SNPs in the *MTHFR* gene, A2756G in *MTR*, A66G in *SHMT1*, G1958A in *MTHFD1* and 844ins68 in *CBS* in genetic susceptibility to aggressive non-hodgkin's malignant lymphoma in the west-Siberian region. **Methods.** Eighty nine unrelated patients from the Novosibirsk City Haematological Center with aggressive NHL were investigated. Genomic DNA was isolated from leukocytes in venous blood and also from buccal epithelium, using the standart methods of DNA separation. A PCR-restriction fragment length polymorphism (RFLP) assay was used to detect the *MTHFD* G1958A and *CBS* 844ins68 SNPs. Genotiping of the *MTHFR*, *MTR*, *MTRR* and *SHMT1* gene SNPs was carried out by real-time PCR allelic discrimination with TaqMan probes. The alleles and genotype distribution of SNPs in patiens were compared with the distribution in healthy, white Russian subjects from Novosibirsk. **Results.** We determined the allele and genotype frequencies for the C677T and A1298C SNPs in the *MTHFR* gene, A2756G in *MTR*, A66G in *SHMT1*, G1958A in *MTHFD1* and 844ins68 in *CBS* gene in the NHL case and control groups. For all these SNPs, the genotype frequencies were in Hardy-Weinberg equilibrium in the control group. The polymorphisms G1958A *MTHFD1* and C1420T SHMT1 showed significant assotiation with aggressive NHL. Allele *1958A MTHFD1* was associated with decreased risk of diffuse large B-cell lymphoma (OR=0,429; C. I. [0. 279-0. 659], p<0,00008). Allele 1420T *SHMT1* was associated with increased risk in the group of another large B-cell non-hodgkin's lymphoma (OR = 1,862; C. I. [1,073 - 3,231], p < 0,026). Any association with indolent non-hodgkin's lymphoma was not revealed. **Conclusions.** Thus, single nucleotide polymorphisms G1958A *MTHFD1* and C1420T *SHMT1* may play a role in pathogenesis of non-hodgkin's malignant lymphoma. The bouth SNPs may cause to NHL through creating an imbalance between entities in folic acid metabolism.

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ANTI-TUMORAL EFFECT OF SODIUM METAARSENITE (KML001) IN NON-HODGKIN'S LYMPHOMA WITH BREAKING-DOWN THE RESISTANCE OF ADRIAMYCIN

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Background. Arsenic compounds have been used in traditional medicine for several centuries. Especially, arsenic trioxide (As_2O_3) has been proven effective in acute promyelocytic leukemia, and also has been tried for the treatment of other hematological malignancies, including non-M3 type acute myelocytic leukemia, multiple myeloma. A drawback of As_2O_3 is to be administered intravenously. Sodium metaarsenite ($NaAs_2O_3$: code name KML001) is an orally bio-available arsenic compound with potential anti-cancer activity. However, the effect of KML001 has not been well studied in non-Hodgkin's lymphoma (NHL). **Aims.** Firstly, to determine the anti-tumoral effect of KML001 in NHL, and secondarily, to investigate the mechanism of anti-tumoral effect of KML001 in Jurkat and Jurkat R (adriamycin resistant cell) cell line. **Methods.** Eleven NHL cell lines were used in this study including Jurkat R cells. Cellular inhibition was measured by MTT assay. Expression of molecules was done by western blot. Analysis of cell cycle was used by flow cytometry. Transcriptional expression of catalytic subunit of telomerase, hTERT, and telomere length were done by real-time PCR. *In vivo* anti-tumoral effect was observed in nude mouse (Balb/c-nu/nu mouse) after inoculation of NHL cell line (Daudi). **Results.** KML001 inhibited the cellular proliferation in all NHL cell lines (IC_{50} of 5 x 10^{-8}M) as well as JurkatR cells (IC_{50} of 1 x 10^{-8}M) in a dose-dependent manner. KML001 (1 x 10^{-7}M) was induced G1 arrest which was associated with decreased expression of cyclin B1, cyclin E1, CDK1, CDK2, CDK4, and CDK6, and reduced kinase activities of CDK4 and CDK6 in Jurkat and JurkatR. In addition, KML001 increased the p21 and p27 levels in Jurkat and JurkatR cells. Expression of Bcl-2, Bcl-$_{XL}$, Mcl-1, proform of caspase-3, caspase-8, and caspase-9 was decreased, in contrast, expression of PARP was increased in Jurkat and JurkatR cells by KML001. Regarding the intracellular signaling, KML001 inhibited the activation of pSTAT1, 3, 5, NF-κB, pAKT, p-mTOR, p-GSK3 ß and pERK, while expression of pPTEN, pp38 and pJNK was up-regulated in Jurkat and JurkatR. Real-time PCR with RNA extracted from KML001-treated Jurkat and JurkatR cells showed a reduction of catalytic subunit of telomerase, hTERT, associated with a shortening of the telomere length. Furthermore, DNA damage molecule (γ-H2AX) was increased in KML001-treated in Jurkat and JurkatR cells. *In vivo* anti-tumoral activity of KML001 in a xenograft model of lymphoma cell line (Daudi) showed that tumor burden was significantly reduced (P < 0. 01) 42 days after oral feeding of KML001. Especially, *in-vivo* anti-tumoral effect of 3. 5 mg/Kg KML001 was comparable to that of doxorubicin (2. 5 mg/Kg, P < 0. 05). **Summary and Conclusions.** KML001 demonstrated anti-tumoral effect via various mechanisms including cell cycle arrest, induction of apoptosis, and inhibition of JAK/STAT, PI3K and MAPK pathways as well as targeting the telomere with DNA damage. Also, our results suggested that KML001 may overcome the resistance of chemotherapeutic agents. Furthermore, the sizes of tumor nodules in nude mice were reduced by KML001. Collectively, KML001 may be a candidate agent for the treatment of *de novo*, refractory and relapsed NHL.

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EVIDENCE FOR REGULATION OF APOPTOSIS BY LMP1 ONCOPROTEIN OF EPSTEIN-BARR VIRUS IN PATIENTS WITH LOW GRADE B-CELL LEUKEMIC LYMPHOMAS

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Background. In all EBV - associated malignancies, the virus displays a latency program of infection, which represents a restricted pattern of gene expression. Among the products of its genes, latent membrane protein 1 (LMP1) has been characterized as a potent transforming protein. LMP1 is expressed at widely different levels in cells of a single clone, a fact that may explain the stimulation of multiple distinct pathways, such as oncogenesis, cytotoxicity, and apoptosis. Studies in LMP1-positive cell lines have shown the coexisting apoptotic properties of LMP1. Survivin, a member of the inhibitor of apoptosis (IAP) family, is suggested to be an important regulator of the mitochondrial apoptotic pathway. The literature lacks information about the expression of LMP1 in the peripheral blood of patients with non-EBV-related lymphoproliferative diseases. **Aims.** To examine the apoptotic properties of LMP1 oncoprotein, in patients with low grade B-cell lymphomas, by studying the expression of survivin. **Patients and Methods.** Fifty-nine (59) patients with leukemic low grade B-cell lymphomas were included in the study. Informed consent was obtained from all patients. The patients' distribution to the individual lymphoproliferative diseases was as follows: chronic lymphocytic leukemia: 37, marginal zone lymphoma: 14, mantle cell lymphoma: 4, hairy cell leukemia: 2, follicular lymphoma: 2. Thirty-eight (64. 41%) patients were treatment naïve, while the rest had not been treated for the last 6 months We used, as a control group, 30 EBV-negative healthy adults. Peripheral whole blood specimens were obtained. Genomic DNA (QIAamp® DNA Blood mini kit - Qiagen) and RNA (Trizol protocol - Invitrogen, Carlsbad, CA) were extracted within 6 hours of collection. For the detection of EBV, a quantitative real time (qRT) PCR was performed for the BXLF-1 gene by using the EBV R-gene® Quantification COMPLETE kit (Argene). RNA from EBV-positive patients was tested by qRT-PCR, while RNA from all patients was tested by qRT-PCR to determine the levels of survivin and ABL expression. Primers and probes for the genes of interest, *LMP1, ABL*, *survivin* were chosen and qRT-PCR was carried out for each individual gene of each sample on LightCycler® 2. 0 (ROCHE, Mannheim, Germany). All reactions were performed twice. Relative gene expression (survivin vs. ABL) between normal and study blood samples was calculated by using the $2^{-\Delta C_T}$ method. Mann-Whitney U test was used for the statistical analysis of the results. **Results.** Twenty-five out of 59 patients (42. 37%) were EBV-positive, and 20 of them (80. 0%) expressed LMP1 (33. 89% of all patients). The levels of survivin were 4. 35 times higher in EBV-negative in comparison to EBV-positive patients (2-tailed p=0. 003), moreover they were 9. 2 times higher in LMP1-negative versus LMP1 positive patients (2-tailed p=0. 005). **Conclusions.** LMP1 expression in our patient series is higher (33. 89%) from the rates already reported in the literature. In our study, LMP1-positive patients express lower levels of survivin (p=0. 005), and this finding is in accordance to the hypothesis that LMP1 oncoprotein can induce apoptosis. We speculate that the apoptotic effect of LMP1 may be more profound in patients with low grade B cell lymphomas that are not causally correlated with EBV.

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SERUM PROTEOMIC PROFILE IN PATIENTS INVOLVED BY DIFFUSE LARGE B CELL LYMPHOMA (PRELIMINAR REPORT)

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Background. Diffuse Large B-Cell Lymphoma (DLBCL) is the most frequent subtype of Non-Hodgkin Lymphoma in the world (35%). Despite of R-CHOP chemotherapy, patients with DLBCL continue to relapse/refractory after treatment. For this reason, the search for biomarkers that could predict complete response (CR) and survival is important. Proteomics is in charge of the identification, quantification and characterization of the proteins in tumor cells. There are some descriptions about the proteomic profile of lymphomatous cellular line, but there are not any reports about the proteomic profile in serum in patients with DLBCL. **Aims**. We present the preliminary results from the serum profile using proteomic technology in patients involved by DLBCL. **Methods**. The serum from 18 patients with diagnosis of the novo DLBCL who were treated with R-CHOP and 8 healthy people (control cases) were analyzed. The technology Proteominer® was used. Proteins were separated in double dimension gels (2D-SDS-PAGE at 12%) and stained with Coumassi/silver. Spot lecture was done by the software PDQuest. The potential spots were cut and analyzed by Liquid Chromatography Mass Spectrometry. Clinical variables were also analyzed together with the proteomic profile results. **Results**. There were 8 proteins identified with differential expression in the serum of patients with DBCL, 2 of the inflammatory type (amyloid A and P) and antioxidant (GPX3). GPX3 was the most frequent protein observed in 72% of the cases, compared with the control group where was only observed in 12% (p=0. 009). This protein presented a sensitivity of 72% (CI95%: 49% to 85%), specificity of 87. 5% (IC95%: 63-98%) with a PPV of 93% (CI95%

68% to 99%) and NPV of 58% (CI95% 32 to 81%). The clinical characteristics of patients with GPX3 (+) compared with the GPX3 (-) were the followings: More frequent in males (54% vs 20%, respectively), older age, elevated LDH (64% vs 40%, respectively), worst ECOG (23% vs 0%,), advanced IPI score (intermediate and high; 46% vs 20% and 15. 4% vs 0%, respectively). Regarding overall rate response (ORR), that was higher in patients with GPX3 (+) vs. GPX3 (-). (100% vs. 66%, respectively p=0. 07). **Conclusions**. The proteomic profile at the serum level from patients with DLBCL has an inflammatory and antioxidant component in serum. The GPX3 was associated with variables of poor prognosis for DLBCL. GPX3 showed to be a potential biomarker for predicting ORR. Therefore, in our group we are evaluating the role of plasma GPX3 by ELISA in patients with DLBCL to attempt corroborate this data.

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GENOMIC IMBALANCES IN HCV-RELATED LYMPHOMAS ARE SIGNIFICANTLY CLUSTERING IN HIGH GRADE MALIGNANCIES

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Background. In HCV-related lymphomas, HCV supports mono- oligo- clonal B-lymphoproliferation, thus favouring selection of malignant clones. A role of HCV in pathogenesis is further documented by low grade lymphoma regression under antiviral treatment. However, as genetic damage accumulates, low grade lymphoma may evolve into a diffuse large B-cell tumour or a high-grade malignancy may be present from the beginning in the form of an aggressive B lymphoma. Genetic studies on these malignancies are still scarce. Using FISH and CGH in a series of 16 HCV positive patients we found a prevalence of trisomy 3q in low grade lymphomas and of 2q deletion in diffuse large B cell lymphoma (Matteucci et al Leukemia 2008;22:219-222). In 12 cases of HCV related splenic marginal zone lymphomas Novara et al (Hum Pathol 2009;40:1628-37) found recurrent deletions at chromosome 7q, 8p, 13 and 17p and recurrent duplications involving chromosome 17q, 18q, and Xp. A 3q duplication was confirmed in only one case. **Aims.** To increase knowledge of genomic changes in low- and high- grade HCV related Lymphomas. **Methods.** In a series of 18 patients with lymphomas histopathology showed marginal zone lymphoma (MZL, low grade lymphoma)in 5, diffuse large B-cell lymphoma (DLBCL, high grade) in 7, follicular lymphoma (FL) in 3, Hodgkin's lymphoma in 2 and multiple myeloma in 1. In all cases HCV infection was serologically demonstrated. Paraffin embedded tissue sections from pathological lymph nodes were used for DNA extraction in 17/18 cases. Peripheral blood cells were used for the other (DLBCL). Our analysis was based on Comparative Genomic Hybridization as the best technique in our hands to analyze genomic imbalances on archival samples. **Results.** A total of 71 imbalances (40 gains and 31 losses) were found. Most (28 gains and 16 losses) were found in high grade DLBCL, as expected. Two or more gains involved chromosomes 3, 5, 6, 7, 8, 9, and 17q, while losses were found at chromosomes 2, 6, 8, 9, and 17. Only 2 losses (10 q and 14q) were found in the 5 cases of MZL. Nine imbalances (4 gains at chromosomes 2, 8, 16, and 18) and 5 losses at chromosomes 1, 10, 16, 19, and 22 were found in 3 cases of follicular lymphoma). Overall 14 imbalances (5 gains at chromosomes 1, 6, 9, 14, and 21 and 9 losses at chromosomes 1, 2, 8, 10, 12, 16, 17, 19, and 20) were detected in multiple myeloma, while only normal results were obtained in the 2 samples of HD. **Conclusions.** Two isolated losses in MZL involved chromosome 10q and 14q, confirming ours and Novara's previous observations. A 10q loss was also found as an isolated change in one case of FL, thus emphasizing this lesion as an early event. A significantly higher genomic complexity (p<0. 05, Mann-Whitney test) emerged in high-grade lymphoma. Only 1 out of the 7 cases was normal. Interestingly, a 2q loss was confirmed in two DLBCL, and both cases showed concomitant 3q gain and a past history of low grade lymphoproliferation (Matteucci, Leukemia 2008).

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99MTC-TIOGLUCOSE AS A POTENTIAL IMAGING AGENT IN NON-HODGKIN LYMPHOMAS

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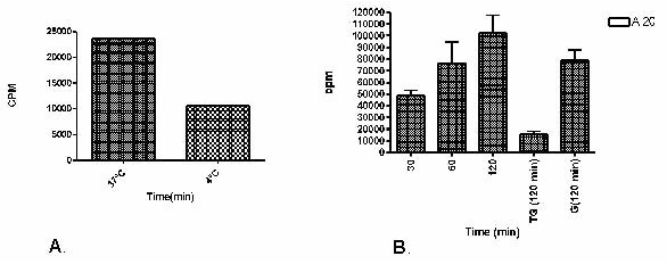
Background. Glucose metabolism has been shown to be increased in neoplastic cells. This altered metabolism confers them a selective advantage for survival and proliferation. Glucose analogs (eg. ^{18}FDG) are used to evaluate this increased metabolic activity in different neoplasms and its role has been validated in certain types of lymphomas. Obtaining glucose analogs labeled with ^{99m}Tc would be of interest, provided its low cost and high availability. **Aims.** Development and biologic evaluation of 1-tio-β-D-glucose (1TG) -^{99m}Tc. Evaluation as imaging agent in non-Hodgkin lymphomas (NHL). **Methods.** 2 mg of 1TG were added to 40 ul SnCl2 (80ug/ml) and 370 MBq $^{99m}TcO_4^-$ and incubated during 30 min at room temperature. *In vitro* stability of ^{99m}Tc-1TG was evaluated during 24 h. Radiochemical purity and stability were evaluated by means of different chromatographic systems: ITLC-SG, FM: MEK, NaCl 0. 9% and HPLC. *In vitro* biological evaluation was performed in A20murine NHL cells: blockage assays using 1TG and cold glucose were made. Internalization assays were evaluated at 4 and 37ºC. Biodistribution was assessed at 1 and 2 h in normal BALBc mice (n=3). **Results.** radiochemical purity of 1TG-^{99m} Tc was > 95% and remained stable during 24 h. A20 cell assays showed increased uptake of ^{99m}Tc-1TG at 2 h and blockage by the addition of cold 1TG. This uptake was not modified when cold glucose was used. Internalization studies at 4 and 37ºC suggest that ^{99m}Tc-1TG uptake is performed by active transport. Biodistribution at 1 and 2 h showed high blood clearence and renal elimination. Conclusions. Labeling of 1-TG - ^{99m}Tc was performed with high radiochemical purity and stability. Biological evaluation is that of an imaging agent. This results support the hypothesis that 1-TG - ^{99m}Tc may have a potential use as imaging agent in NHL and encourage further studies in animal models, which are now in courseì.



A. Internalization of 99mTc-TG in A20 cells
B. Internalization and blockage study (glucose and TG)

Figure 1.

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SYNERGY OF PRO-APOPTOTIC DRUGS IN SEZARY LYMPHOMA

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Background. Sezary syndrome is a rare cutaneous T-cell lymphoma (CTCL) of poor prognosis characterized by erythroderma, lymphadenopathy and the presence of circulating Sezary cells. Current treatments are often associated with a partial and transient response, justifying the evaluation of new therapies. Expression profile studies have identified gene targets of potential interest in therapy of peripheral T-cell lymphomas. The HDACi suberoylanilideacide hydroxamic (SAHA) causes cellular death by apoptosis and cell cycle arrest of a wide variety of transformed cells. The treatment of SAHA refractory CTCL patients has a response rate of only 30%, suggesting the need for a combined chemotherapy approach. The MithramycineA inhibits the activity of transcription factors of the Sp3 family and modifies the expression of genes involved in apoptosis, angiogenesis and cell survival. Mithramycin A (MM-A) has not been evaluated in CTCL. **Aims.** The purpose of this study was to evaluate the combined effects of Saha + MM-A on Sézary cells. **Methods.** Sezary cells were obtained from four patients, the lineage Hut-78 cell line derived from the ATCC (Rockville, MD, USA). Activated CD4 + T-lymphocytes stimulated by IL-7 + anti-CD28 were chosen as the normal counterpart for Sézary. Resistant clones of Hut-78 were obtained by limiting dilution or by culture in progressive concentrations of Saha / MM-A. Effects of these drugs on cells were evaluated for proliferation, survival / viability, apoptosis and the cell cycle by FACS analysis, MTT test with Annexin V-7AAD and propidium iodide. The study of synergies between drugs was made by serial dilution of ED50 and analysis using the CalcuSyn (method of Chou and Talalay). Gene expression and miRNA profiling were performed on Agilent platform. **Results.** The treatment of Sezary cells by Saha and MM-A induced a dose an time-dependent apoptosis, with a synergistic effect of the two drugs. The normal CD4 + T cells activated by IL-7 + anti-CD28 were less sensitive to Saha and MM-A. Clones resistant to Saha, MM-A or both drugs were obtained and will be used in vitro model of resistance to cytotoxicity. **Conclusions.** The analysis of the transcriptome od cells exposed to cytotoxic drugs may specify the different path-

ways involved in response / resistance of Sezary cells to SAHA / MM-A. We focused our attention on the expression of genes involved in apoptosis, proliferation, cell cycle, the JAK / STAT pathway, the family of TNF-R, interleukins and their receptors, and the TCR siganling, these genes being identified as targets of SAHA.

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AQUAPORIN-4 EXPRESSION IN PRIMARY HUMAN CNS LYMPHOMAS CORRELATES WITH TUMOR CELL PROLIFERATION AND HETEROGENEITY OF THE VESSEL WALL

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Background. No literature data are available concerning the expression of aquaporin-4 in primary central nervous system lymphomas and the relationship between aquaporin-4 expression and the morphological characteristics of blood vessels. **Aims and Methods.** We have investigated this relationship in 24 human diffuse large B-cell primary central nervous system lymphomas by means of immunocytochemistry and confocal laser microscopy. **Results.** i) A high aquaporin-4 expression correlated with a high Ki-67 index and aquaporin-4 marked tumor and endothelial cells in cytoplasm and plasma membranes, while aquaporin-4 expression was low in tumor areas with a low Ki-67 index where few tumor cells were positive to aquaporin-4, and endothelial cells showed aquaporin-4 expression on their abluminal side. ii) Different type of cells participated to vessels formation: $CD20^+$ tumor cells and factor $VIII^+$ endothelial cells; $aquaporin\text{-}4^+$ tumor cells and $CD31^+$ endothelial cells; $CD20^+$ and $aquaporin\text{-}4^+$ tumor cells; glial fibrillary acidid $protein^+$ endothelial cells surrounded by glial fibrillary acidic $protein^+$ tumor cells. **Conclusions.** Overall, these data suggest the importance of aquaporin-4 in primary central nervous system lymphomas due to its involvement in cerebral edema formation and resolution and tumor cell migratory activity, and have documented that tumor microvasculature in lymphomas is extremely heterogeneous, confirming the importance of neoangiogenesis in the pathogenesis of lymphomas.

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DECREASED EXPRESSION OF CXCR4 CHEMOKINE RECEPTOR IN BONE MARROW AFTER CHEMOTHERAPY IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA IS A GOOD PROGNOSTIC FACTOR

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Background. Chemokines and their receptors have been proved to be involved in cancer progression. CXCR4 chemokine receptor is widely expressed in normal tissues and plays an important role in development, mobilisation of haematopoietic stem cells, and trafficking of lymphocytes. CXCR4 is constitutively expressed on normal and malignant B lymphocytes derived from patients with B-cell lymphoproliferative disorders and has significant role in cell migration to lymph nodes and bone marrow. Overexpression of CXCR4 has been shown in thyroid and colon cancer. In breast cancer expression of CXCR4 is correlated with cancer metastases. Non-Hodgkin's lymphomas (nHL) constitute heterogeneous group of lymphoproliferative diseases, with different presenting features, clinical course and response to treatment. In Western countries majority of nHL's are B-cell origin. Lymphoma cells can localize not only to lymph nodes, but also can migrate to peripheral blood and metastase to other organs, including bone marrow. **Aims.** The purpose of this study was to determine CXCR4 gene expression in peripheral blood and bone marrow of non-Hodgkin's lymphoma patients before and after treatment. **Methods.** Samples of peripheral blood and bone marrow aspirates of 20 patients (9 females, 11 males; aged 26-73, median age 57 years) with B-cell nHL (7 follicular lymphoma, 4 mantle cell lymphoma, 3 small lymphocytic lymphoma, 3 marginal zone lymphoma, 2 diffuse large B-cell lymphoma, 1 Burkitt lymphoma) were taken at diagnosis and after chemotherapy. Gene expression was determined by the reverse transcription (RT)-polymerase chain reaction method. Expression was estimated from 0 AU (no amplificate signal) to 3 AU (maximal amplificate signal). Statistical analysis was performed using Wilcoxon and Cox test ($p<0,05$). **Results.** We observed high level of CXCR4 expression in most patients before treatment: in bone marrow: 3 AU - 10 pts, 2 AU - 8 pts, 1 AU - 2 pts. In peripheral blood: 3 AU - 14 pts, 2 AU - 4 pts, 1 AU - 1 pts, 0 AU - 1 pts. After chemotherapy significant decrease in CXCR4 expression was observed. Bone marrow: 3 AU - 5 pts, 2 AU - 7 pts, 1 AU - 5 pts, 0 AU - 3 pts ($p=0,03$). Peripheral blood: 3 AU - 2 pts, 2 AU - 6 pts, 1 AU - 10 pts, 0 AU - 2 pts ($p=0,0002$). There was a good response to treatment in patients with significant decrease of CXCR4 expression in bone marrow after treatment. Those patients had 10-fold lower risk of death ($p=0,03$). **Conclusions.** Decrease in CXCR4 expression in bone marrow of nHL patients after chemotherapy can be a good prognostic factor.

Table 1.

CXCR4 expression	Peripheral blood (before treatment) - N° of pts	Peripheral blood (after treatment) N° of pts	Bone marrow (before treatment) N° of pts	Bone marrow (after treatment) N° of pts
3 AU	14	2	9	5
2 AU	4	6	8	7
1 AU	1	10	2	5
0 AU	1	2	1	3

1658

EVALUATION OF CD99 AS A PROGNOSTIC MARKER IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH RITUXIMAB INCLUDED IMMUNOCHEMOTHERAPY

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Background. CD99 immunoreactivity has been documented in a variety of tumors, including lymphoid malignancies such as anaplastic large-cell lymphomas. However, few studies have investigated the prognostic impact of CD99 in diffuse large B-cell lymphoma (DLBCL). Lee et al reported that DLBCL with positive CD99 had inferior event-free survival (EFS) in a previous study (Acta Haematol 2011; 125:167-174), but this study included only 13. 7% of patients who were treated without rituximab included immunochemotherapy and needs re-evaluation. **Aims.** The purpose of this study is to evaluate prognostic impact of CD99 in patients with diffuse large B-cell lymphoma (DLBCL) treated with the standard immunochemotherapy, rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP). **Methods.** Patients were included if they were diagnosed as DLBCL by a histologic confirmation, at least 18 years of age and with a complete set of clinical data and adequate paraffin-embedded biopsy specimen for immunohistochemistry (IHC) for CD10, MUM-1, BCL-6, and CD99. All of the patients were treated with 3-weekly R-CHOP immunochemotherapy. Using conventional paraffin embedding, immunoperoxidase staining and tissue microarrays, we retrospectively investigated CD99 expression in 72 DLBCL patients. **Results.** Patients were classified either germinal center B-cell (GCB) type (n = 39) or non-GCB type (n = 24) of DLBCL by classification proposed by Muris et al. There was no difference of event-free survival (p = 0. 769) and overall survival (OS; p = 0. 547) in the whole patient population. Among patients with GCB type, patients with positive CD99 showed superior EFS (2-year EFS 90. 9% vs. 55. 7%, p = 0. 016) and OS (2-year OS 66. 7% vs. 63. 8%, p = 0. 024), compared to those with negative CD99. On the contrary, among patients with non-GCB type, patients with negative CD99 showed superior EFS (2-year EFS 19. 0% vs. 60. 0%, p = 0. 036) and OS (2-year OS 33. 9% vs. 80. 0%, p = 0. 012), compared to those with positive CD99. **Summary and Conclusions.** Prognostic value of CD99 was absolutely contrary to each other according to Muris classification: it is good prognostic factor in patients with GCB type of DLBCL and vice versa.

1659

LACK OF ASSOCIATION OF HHV-6 WITH LEUKEMIC LOW GRADE B-CELL LYMPHOMAS

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Background. Human herpesvirus 6 (HHV-6) is a member of the herpesviridae and was first isolated and characterized from patients with lymphoproliferative

disorders. Like other herpesviruses, it remains latent in certain cells of the immune system after primary infection, and can be reactivated in immunocompromised hosts. Its DNA integrates into the human genome. Due to this property, the oncogenicity of the virus has been speculated early after its first detection. The pathogenetic role of HHV-6 in lymphoproliferative diseases has been of continued interest for the last 20 years. Several studies have been performed to shed light on this correlation. Their conflicting results are due to the different methods used to detect the virus, the diversity of specimens used for this purpose, as well as the wide spectrum of diseases under study. AimsThe aim of the study is to investigate the postulated correlation of HHV-6 infection with leukemic low grade B-cell lymphomas. Patients and MethodsForty-eight (48) patients with leukemic low grade B-cell lymphomas were included in the study. The distribution of the patients at the individual lymphoproliferative diseases was as follows: chronic lymphocytic leukemia 56. 25%, splenic marginal zone lymphoma 27. 08%, mantle cell lymphoma 8. 33%, hairy cell leukemia 4. 17%, and follicular lymphoma 4. 17%. Peripheral whole blood specimens were collected and genomic DNA was extracted by using QIAamp® DNA Blood mini kit (Qiagen). Quantitative real time PCR (QR-PCR) was performed for the U57 gene of HHV-6 by using the CMV HHV6,7,8 R-gene® Quantification COMPLETE kit (Argene). The study was performed on LightCycler® 2. 0 (Roche, Mannheim, Germany). The detection limit of the technique is <156 copies/mL. ResultsThe median age of the patients was 73 years (51-87). The male to female ratio was 1. 1. None of the examined specimens was found positive for HHV-6. ConclusionsThe prevalence of HHV-6 in healthy individuals from Greece has not yet been determined, but we are investigating the prevalence of the virus in blood donors. Studies from other parts of the world give a wide range (2. 9% in the United Kingdom to 40% in Spain) of HHV-6 DNA detection by PCR in the whole blood of healthy individuals, mostly blood donors. Our results support a lack of contribution of HHV-6 in the malignant transformation of leukemic low grade B-cell lymphomas, a result that is compatible with the results of another recent study from Spain.

1660

PRAME EXPRESSION IN NON-HODGKIN'S LYMPHOMA

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PRAME (preferentially expressed antigen of melanoma) expression was determined in 63 cases with non-Hodgkin's lymphoma (NHL). Age range was between 18-79, male/female ratio was 35/28. Thirty five of the cases had diffuse large B cell leymphoma (DLBCL), 20 had low grade lymphoma (LGL), 6 had peripheral T cell lymphoma (PTCL) and 2 had mantle cell lymphoma (MCL). PRAME expression was detected in 21 of the cases; 10 of these cases had DLBCL, 7 had LGL and 4 had PTCL. PRAME expression was not found in 42 cases; 25 had DLBCL, 13 had LGL, 2 had PTCL and 2 had MCL. Totally 10 of the 35 cases with DLBCL and 7 of the 20 LGL had PRAME expression, respectively. No response to initial treatment and/or relapse occurred in 7 of 21 PRAME (+) cases and 16 of 42 PRAME (-) cases. Interestingly, central nervous system involvement occurred in 3 cases without PRAME expression while none of the cases with PRAME expression had central nervous system involvement. Progession free and overall survivals were compared in cases with or without PRAME expression.

1661

FAIRLY PATIENTS TREATED WITH AGGRESSIVE CHEMOTHERAPY

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Background. Anthracyclyne-based chemotherapy regimens are the standar therapy for patients with diffuse large B-cell lympgoma (DLBCL), but such regimens may be poorly tolerated in frail patients (age >65 years, antecedents of cardiac disease oir abnormal ventricular left function). **Aims.** We analyzed the feasibility of a regimen that include pegylated liposomal doxorubicin (PLD) instead of doxorubicin in a CPOP regimen in untrated frail patients. **Results.** sixty-nine patients weren enrolled, in an intent-to treat analysis all were evaluable for efficacy and toxicity. Complete response was achieved in 50 cases (72%), three patients developed severe toxicity and were excluded, nine patients relapse . Thus,actuarial curves at 5-years showed that relapse-free survival was 78 % (95% Connfidence interval(CI) 71% to 84 %) and overall survival was 79% (95% CI: 70% to 85%). Two patients suffer myocardial infarctation, 3 and 6 years after treatment. **Conclusions.** These results, suggests that CPOP appears to be an acceptable alternative for frail patients with DLBCL.

1662

PROGNOSTIC IMPACT OF BCL6 GENE REARRANGEMENT IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background. The *BCL6* gene rearrangement is the most common genetic abnormality in diffuse large B-cell lymphoma (DLBCL), occurring in approximately 35% cases. The prognostic significance of the *BCL6* rearrangement in DLBCL has remained uncertain despite previous studies. **Aims.** The aim of study was investigating the frequency of *BCL6* rearrangement and BCL6 protein expression in two opposite groups of patients DLBCL with favorable and poor outcome. **Methods.** We retrospectively studied *BCL6* rearrangement by fluorescence in situ hybridization with break-apart probes and BCL6 protein expression by immunohistochemical staining of 56 paraffin-embedded specimens from patients (pts) DLBCL - 37 pts with relapse\refractory disease (poor prognosis - group 1) and 19 pts in complete remission more than 3 years (favorable prognosis -group 2). **Results.** In group 1 (37 pts) 60% were male, median age was 48 (range 15-81), 78% had advanced stages and 51% B-symptoms, > 1 extranodal sites involved 51% pts, 35% had received prior rituximab-containing therapy. After median follow-up of 32 months, median progression free survival was only 5 months, overall survival - 14 months. In favorable group 2 (19 pts) 58% were male, median age 46 (range 19-76), 84% had early stages. All pts in this group are alive without disease. *BCL6* rearrangement was detected in 84% of cases in poor prognosis group and only in 21% of cases in favorable group (p<0,001). BCL6 protein expression was not predictive of outcome in neither group and determined respectively 49% and 53% cases. No association was found between gene rearrangement and BCL6 protein expression. **Conclusions.** *BCL6* gene rearrangement correlated with high-risk clinical feature and was found to be an unfavorable prognostic marker in DLBCL. BCL6 protein expression was not associated with gene rearrangement and outcome of disease. Prospective analysis will be needed to clarify prognostic significance of chromosomal abnormality in DLBCL.

1663

CLINICAL FEATURES AND TREATMENT OUTCOMES OF PRIMARY BREAST LYMPHOMA; MULTI-INSTITUTIONAL ANALYSIS OF 63 PATIENTS IN KOREA

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Background. There has been sparse information about primary breast lymphoma (PBL). Recently, several studies suggested some common features: predominant diffuse large B-cell lymphoma (DLBCL) histology, significant risk of central nervous system (CNS) relapse. **Aims.** The aim of this study is to investigate the clinical features and outcomes of PBL and also to find the factors associated with CNS relapse. **Methods.** We analyzed the data of 63 patients diagnosed with PBL from 1994 to 2009, who were identified from our nationwide sur-

vey. **Results.** The median age was 45 years (range, 20-73) and all were female. The Ann Arbor stage was I(37, 59%)/II(22, 35%)/III(4, 6%). ECOG performance status was 0 or 1 in 58 patients (92%) and serum LDH level was elevated in 23 (37%). Thus, the International Prognostic Index (IPI) was mainly low (50, 79%). The most common subtype identified was DLBCL (49, 78%), with the second most common subtype being extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT; 6, 10%). Follicular lymphoma was identified only in one. T-cell lymphoma consisted of peripheral T-cell lymphoma, unspecified (PTCL-U; 4, 6%) and anaplastic large-cell lymphoma (ALCL), anaplastic lymphoma kinase (ALK) positive (1, 2%); ALCL, ALK negative (2, 3%). T-cell lymphoma had significantly more unfavorable characteristics such as presence of B symptom and poor performance status. Thus, the proportion of patients with low-intermediate/high-intermediate IPI was greater in the T-cell lymphoma group, although this was statistically nonsignificant (P=0. 095). The majority of patients (91%) received systemic chemotherapy ± radiotherapy as a curative treatment. Primary surgical resection ± axillary node dissection was performed in 29 patients (46%). Five of the 6 patients with MALT lymphoma were only treated with surgical resection. With a median follow-up of 49. 6 months (range, 4. 4-186. 0), estimated 5-year PFS and OS was 53. 1% (95%CI, 45. 9-60. 3) and 64. 8% (95%CI, 57. 8-71. 8). The PFS and OS of the B-cell lymphoma were significantly better than those of the T-cell lymphoma (PFS, 57. 3% vs 17. 9%, P=0. 007, respectively; OS, 71. 3% vs 17. 9%, P=0. 001, respectively). In multivariate analysis for OS, T-cell phenotype (hazard ratio [HR], 3. 41; 95% CI, 1. 12-10. 31) and low-intermediate/high-intermediate IPI (HR, 3. 89; 95% CI, 1. 26-12. 06) were independent prognostic factors for worse OS. Twenty-one patients experienced progressive disease following first-line therapy. Of these, 7 experienced CNS relapse, and cumulative incidence of CNS relapse at 3-year was 13. 1% (95% CI, 8. 5-17. 7). In multivariate analysis, serum LDH level was only independent predictor for CNS relapse (HR, 10. 31; 95% CI, 1. 20-88. 65). Interestingly, six patients (10%) received prophylactic intrathecal chemotherapy using methotrexate, and there was no CNS progression in these patients. **Conclusion.** DLBCL is the most predominant subtype in agreement with previous series. The survival of patients with B-cell phenotype was better than that of patients with T-cell lymphoma. Distant extranodal failures, especially in the CNS, are a major problem. And, elevated serum LDH level was identified as a risk factor for CNS relapse. Thus, despite the role of CNS prophylaxis are still not clear, it should be considered in PBL patients with elevated serum LDH level.

1664

PRALATREXATE PLUS BEXAROTENE IN PATIENTS WITH RELAPSED OR REFRACTORY CUTANEOUS T-CELL LYMPHOMA (CTCL): STUDY DESIGN AND PRELIMINARY Results FROM AN ONGOING, OPEN-LABEL, PHASE 1 DOSE-FINDING STUDY

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Background. CTCLs are a subset of generally indolent, extra-nodal non-Hodgkin T-cell lymphomas presenting with skin lesions that can disseminate or become aggressive. Patients with early-stage CTCL receive skin-directed therapies, but refractory or late-stage patients require systemic or combination therapy. Pralatrexate is an antifolate approved in the United States for relapsed/refractory peripheral T-cell lymphoma. Pralatrexate demonstrated high activity and acceptable toxicity when used as a single agent in a phase 1 study in 54 patients with relapsed/refractory CTCL; 29 patients were treated at the optimal dose (15 mg/m^2/week for 3 of 4 weeks), with a 45% response rate.[1] **Aims.** Preclinical studies suggest that pralatrexate and bexarotene exert synergistic pro-apoptosis in CTCL cells.[2] The current study was designed to determine the recommended dose, safety, pharmacokinetics, and preliminary efficacy of pralatrexate plus oral bexarotene in patients with relapsed/refractory CTCL. Informed consent was obtained. **Methods.** Eligibility criteria include adult patients with CTCL (mycosis fungoides [MF] ≥ 1B, Sézary syndrome [SS], primary cutaneous anaplastic large cell lymphoma [ALCL]), ≥ 1 prior systemic therapy, and Eastern Cooperative Oncology Group performance status ≤ 2. The intended pralatrexate + bexarotene doses include 15 mg/m^2 + 150 mg/m^2 (Cohort 1), 15 mg/m^2 + 300 mg/m^2 (Cohort 2a), 10 mg/m^2 + 150 mg/m^2 (Cohort 2b), and 10 mg/m^2 + 300 mg/m^2 (Cohort 3). Pralatrexate is administered weekly via intravenous push for 3 of 4 weeks; bexarotene is self-administered orally daily with food; patients also receive vitamin B_{12} and folic acid supplementation. The standard 3 + 3 dose-escalation design is used to determine the maximum tolerated dose (MTD). Cohort escalation occurs upon dose-limiting toxicities (DLTs) in < 33% of patients in any cohort. The MTD is the highest dose with < 33% DLT incidence. The following are considered DLTs if they occur in cycle 1: ≥ grade 3 neutropenia (or granulocyte colony-stimulating factor administered), thrombocytopenia, treatment-related non-hematologic toxicity, hyperlipidemia, or hypothyroidism; and treatment-related adverse events causing bexarotene dose omission for ≥ 10/28 days or pralatrexate dose omission/reduction. **Results.** A total of 14 patients (6 female, 8 male; aged 39-85 years) have been treated (11 with MF, 1 with transformed MF, 1 with ALCL, and 1 with SS). The median number of prior therapies was 3 (range, 2-14). In Cohort 1, 0/3 patients experienced DLTs. In Cohort 2a, 2/3 patients experienced DLTs (≥ grade 3 neutropenia [n = 1] and ≥ grade 3 thrombocytopenia [n = 1]). The MTD was identified as pralatrexate 15 mg/m^2 + bexarotene 150 mg/m^2. Response data are available for 10 patients, as presented in Table 1. **Summary and Conclusions.** The MTD of the pralatrexate + bexarotene combination has been determined. Interim data indicate that the combination has been well tolerated and demonstrated robust activity, with 6 responses (1 complete response [CR]; 5 partial responses [PRs]) among the 10 patients with available response data. Enrollment is ongoing; detailed results, including an expanded cohort of 30 patients treated at the MTD, will be presented in the future.

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Table 1. Interim best response data.

Best Response	CTCL Subtype	Cohort and Dosing Schedule (Pralatrexate + Bexarotene)
CR[a]	Primary cutaneous ALCL	Cohort 1 (15 mg/m^2 + 150 mg/m^2)
PR[a]	MF	Cohort 1 (15 mg/m^2 + 150 mg/m^2)
PR[a]	MF	Cohort 1 (15 mg/m^2 + 150 mg/m^2)
PR[a]	MF	Cohort 1 (15 mg/m^2 + 150 mg/m^2)
PR[a]	MF	Cohort 2a (15 mg/m^2 + 300 mg/m^2)
PR	Transformed MF	Cohort 2a (15 mg/m^2 + 300 mg/m^2)
SD	MF	Cohort 1 (15 mg/m^2 + 150 mg/m^2)
SD	MF	Cohort 1 (15 mg/m^2 + 150 mg/m^2)
SD	MF	Cohort 1 (15 mg/m^2 + 150 mg/m^2)
SD	MF	Cohort 2a (15 mg/m^2 + 300 mg/m^2)

[a]Patient remains on study treatment. SD, stable disease.

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SAFETY AND EFFICACY OF RITUXIMAB AND CHLORAMBUCIL IN COMBINATION AS FIRST-LINE TREATMENT IN PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA PATIENTS

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Background. first line treatment for follicular non Hodgkin lymphoma is still a controversial question. Usually patients needing treatment for this indolent disease receive Rituximab in combination with systemic chemotherapy: R-CHOP, R-CVP, R-Fludarabine and more recently R-Bendamustine are considered treatment of choice. We previously published our experience in low grade non Hodgkin lymphoma using combination of Rituximab plus Chlorambucil achieving approximately 90% of response rate with an manageable toxicity. **Aims.** here we report clinical results achieved in newly diagnosed follicular lymphoma patients considered for receiving systemic treatment. **Methods.** eighty-five patients (32 male, 53 female) with a median age of 58 years, all diagnosed with follicular lymphoma, were considered eligible to receive the combination of Rituximab and Chlorambucil with the following schedule: Rituximab standard dose weekly for four doses and Chlorambucil 6mg/mq for six weeks consecutively (induction phase) then, patients responding received a single dose of Rituximab and Chlorambucil for 14 days each month for an additional four months. **Results.** most patients were considered in stage III-IV (76%), one third of them

presented with bulky disease and B-symptoms were present in approximately 20%. The majority of patients were FLIPI 1-2, while 30% of them had extranodal involvement. After induction phase 97% of patients presented a clinical improvement and of consequence all these patients received the additional monthly treatment. Eighty patients completed the scheduled treatment and were evaluated at the end of therapy: 75% achieved a complete remission, 22% a partial remission. With a median follow up of 40 months, the median disease free survival was not reached. The 5 years disease free survival probability is 73%. **Conclusions.** our results confirm the clinical activity of the combination R-Chlorambucil as previously reported in low grade lymphoma patients. Complete response observed is similar to those reported with more aggressive combination regimens, confirming the relevant role of monoclonal antibody in the treatment of such indolent disease. The combination of R-Chlorambucil is safe and active treatment for newly diagnosed follicular non Hodgkin lymphoma patients and, considering its low toxicity profile, might be offered as valid alternative option for those patients unsuitable for aggressive chemotherapy.

1666

INCIDENCE, CLINICAL MANAGEMENT AND OUTCOME OF PERIPHERAL T-CELL LYMPHOMA: A POPULATION-BASED STUDY IN THE UK

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Background. Peripheral T-cell lymphomas (PTCL) are a heterogeneous and rare group of disorders with approximately 400 cases diagnosed in the UK each year, accounting for nearly 5% of all non-Hodgkin lymphomas. Prognosis is considered to be poor for many patients, however, few population-based studies exist that examine the clinical management and outcome of these diseases. **Methods.** Cases of PTCL (>18 years old) diagnosed 2004-2010 were identified from a specialist population-based register of haematological malignancies - the Haematological Malignancy Research Network (www. HMRN. org). HMRN covers a population of 3. 6 million, and all diagnoses are made at a centralised specialist integrated diagnostic laboratory (www. HMDS. info) and coded to the latest WHO classification. Clinical information including demographic, prognostic, all treatment episodes and response data are routinely abstracted from medical records for each case using standardised abstraction forms and procedures. All patients are linked to the NHS Central Register for accurate survival data.

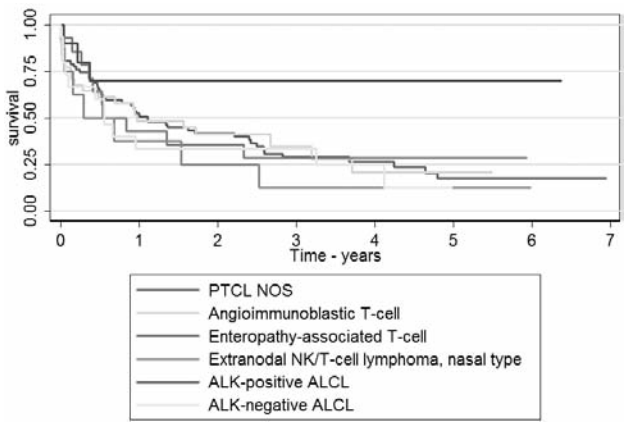

Figure 1. Kaplan Meier Survival Estimates by Peripheral T-cell Lymphoma Subtype.

Results. 150 PTCLs were ascertained over the six year period these comprised; peripheral T-cell NOS (45%), angioimmunoblastic T-cell (21%), ALK-negative anaplastic large cell lymphoma (10%), enteropathy-associated T-cell (9%), ALK-positive anaplastic large cell lymphoma (7%), extranodal NK/T-cell lymphoma, nasal type (5%), anaplastic large cell lymphoma - ALK status unknown (3%). The overall incidence rate was 0. 7 per 100,000 and the European standardised rate was 0. 6 per 100,000 (95% confidence intervals (95%CI) 0. 5-0. 7). Median age at diagnosis was 66. 9 years (range 22. 7-95. 6) and there was a male excess (sex-rate ratio, 1. 4: 95%CI 1. 0-1. 9). 55% of patients were diagnosed with advanced stage disease (III/IV). The majority of patients received chemotherapy as first line treatment (77%) and these were primarily CHOP based regimens (86%). 29% of patients were refractory to first line treatment or relapsed and went on to receive further treatment(s). Of those who had a response after first line treatment, median time-to-progression was 4. 9 months. There was variation in subsequent therapies used; for example at second line treatment 16 % of patients received DHAP, 14% had an autologous stem cell transplant and 13% were given gemcitabine-based regimens. Median survival for all patients was 12 months (range 0-82) and survival at 5-years was estimated to be 25%. There was marked variation in survival by subtype (figure). For those patients who received subsequent treatment(s), response was generally poor with a median survival from time of second line therapy of 9. 9 months (range 1. 4-59. 7). **Conclusions.** PTCLs have no standard treatment approach beyond first line therapy, resulting in heterogeneous treatment pathways. Prognosis is generally poor, and salvage treatment(s) appear to be ineffective for the majority of patients who receive them. HMRN is a large population-based study, which includes trial and non-trial patients, and the findings reflect the reality of the clinical management and outcome of PTCL.

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THE ABSOLUTE MONOCYTE COUNT AT THE TIME OF DIAGNOSI PREDICTS OVERALL SURVIVAL OF PATIENTS WITH MANTLE CELL LYMPHOMA

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Background. There is increasing evidence of the microenvironment influence on the growth of different non-Hodgkin lymphoma (NHL) subtypes. Monocytes and lymphoma-associated macrophages have been shown to be associated with lymphoma cell survival and drug resistance. The absolute monocyte count has been recently reported to be associated with survival of follicular lymphoma and diffuse large B cell lymphoma. **Aims.** To assess the prognostic impact of absolute monocyte count (AMC) at diagnosis in mantle cell lymphoma (MCL) patients. **Patients and Methods.** One hundred forty-two MCL patients were selected out of 2,426 NHL cases recorded on the databases of the Oncology Institute of Southern Switzerland in Bellinzona (Switzerland) and the Division of Hematology of the Ospedale Maggiore della Carità in Novara (Italy), which include patients treated between 1980 and 2011. The AMC at the time of diagnosis was available in 98 cases, which are the object of the present analysis. Median age was 70 years (range 35-94 years); 83% had stage III-IV, 66% had intermediate-high/high International Prognostic Index (IPI) risk and 48% were at high risk according to the sMIPI (simplified MCL international prognostic index). Eighty-five pts (86%) were treated with chemotherapy, 34 (35%) had rituximab, 6 (7%) had front-line autologous stem cell transplantation.

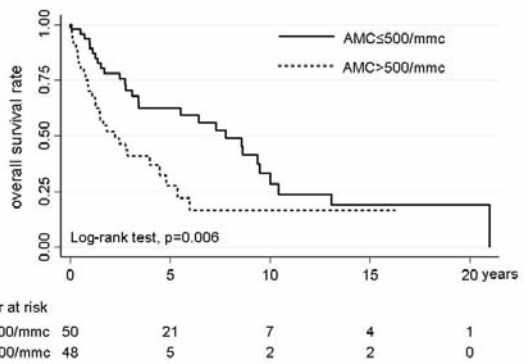

Figure 1.

Results. Median AMC at diagnosis was 500/mmc (range 50-9,000). The best cut-off value of AMC determined by the receiver operating characteristics (ROC) analysis based on dead/alive status was 504/mmc, very close to the median val-

ue, which was then chosen as a cutpoint for subsequent analysis. After a median follow up of 7 years (interquartile range, 3-16), 57 pts (58%) died. Five-year overall survival (OS) was 29% (95%CI, 14-45%) for patients with AMC >500/mmc versus 59% (95%CI, 42-73%) for patients with AMC ≤500/mmc (p 0. 006). In univariate analysis, increased AMC, elevated beta-2 microglobulin, elevated LDH, age>60, poor performance status, and poor-risk sMIPI showed a significant impact on OS. In multivariate analysis (Cox model), AMC >500/mmc at diagnosis remained an independent significant prognostic factor after adjusting for either the sMIPI or the IPI [HR, 2. 6 (95% CI, 1. 2-5. 9)]. **Conclusions.** AMC>500/mmc identifies poor-risk patients in MCL and may provide additional prognostic information over the most commonly used prognostic indices.

1668

IMPACT OF THE MICROENVIRONMENT ON THE PROGNOSIS OF PRIMARY BONE DIFFUSE LARGE B CELL LYMPHOMA

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Background. Primary non-Hodgkin lymphoma of the bone (PLB) is a rare neoplastic disorder, comprising 5% of extranodal lymphomas. Eighty percent of the PLB cases are diffuse large B-cell lymphomas (PBDLBCL). Overall, PLB carries a favourable prognosis. Although the microenvironment has a significant influence on the tumour cell growth, outcome of the disease and response to treatment, PBDLBCL has not been characterized with regards to the infiltrating reactive immune cells. **Aims.** To determine the prognostic significance of tumour cell microenvironment on the outcome of the disease and possible association with the germinal centre-B (GC-B) and activated B cell (ABC) phenotype. **Methods.** Forty-five cases of PBDLBCL diagnosed at the I. Department of Pathology Semmelweis University and Department of Pathology Leiden University were studied. The GC-B versus ABC subtype was determined by Tally's immunostaining algorithm based on the expression of GCET1, CD10, MUM1, FOXP1 and LMO2 antigens. Different components of the microenvironment, such as T cell subsets and macrophages of the microenvironment were analysed by immunohistochemistry using tissue microarrays (TMA). **Results.** A group of 45 patients with PBDLBCL was studied. Twenty-nine patients had single bone infiltration, while multifocal bone involvement (scored as stage IV) was noted in 11 cases. The mean IPI score was 1. 8. The overall survival was 80% with a median follow up of 51 months. Most patients received combination therapy in the form of surgical resection, poly-chemotherapy and radiotherapy. Thirty-two patients (71%) had a complete remission (CR), 9 patients died of the disease (20%), 4 had progressive disease (PD), 5 had partial response (PR) and died during therapy. For 4 patients the outcome was unknown. There was significant association between age and survival: 7 out of 9 patients >60 years of age died of disease. Stage and survival did not show any significant association: overall survival of stage I patients were 83% and 77% for stage IV. The number of tumour infiltrating T-lymphocytes represented by CD3 positive cells showed significant correlation with the outcome and with the phenotype of the tumour cells. Significantly higher number of tumour infiltrating CD8+ cytotoxic T cells and lower proliferation rate were observed in cases with CR compared to the patients with PR or PD disease. **Conclusions.** Our cohort of PBDLBCL patients had favourable outcome with 80% overall survival independent of tumour cell phenotype. Age at disease onset, tumour infiltrating CD3+ T cells and CD8+ cytotoxic T-cells and lower proliferation rate significantly correlated with patients' survival. The finding of a positive impact of tumour infiltrating T-cells in PBDLBCL is in accordance with earlier reports of nodal lymphomas, however our study is the first to show association between microenvironment and disease outcome.

1669

BENDAMUSTINE IN THE TREATMENT OF AGGRESSIVE NON HODGKIN LYMPHOMA. A RETROSPECTIVE ANALYSIS IN SPAIN

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Introduction. Bendamustine is an alkylating-purine analog hybrid agent currently approved in EU for indolent non Hodgkin lymphoma, chronic lymphocytic leukemia and multiple myeloma; however the experience in aggressive lymphoma is limited. **Aims.** The aim of this study is to evaluate the efficacy and toxicity of Bendamustine in patients with aggressive lymphoma. **Methods.** We conducted a retrospective study to analyze the experience in Spain with Bendamustine alone or in combination in patients with aggressive lymphoma. Nine Spanish hospitals have participated in this study. Endpoints were overall response and complete response rate as *IWRv2003 criteria* and toxicity as *CTCAE v3. 0 of NCI scale*. **Results.** Between August 2009 and December 2011 a total of 30 patients with aggressive lymphoma were treated with Bendamustine in nine medical Institutions in Spain. Male/female: 16/14. Median age 76. 6 year (48-88). The histology subtypes were: DLBCL (23) T cell lymphoma (2) and other histology (5). Ann Arbor stage≥3: 25 cases with extranodal involvement in 22. ECOG≥2: 13 patients. Bendamustine treatment: 25 patients received Bendamustine as salvage therapy (relapse after CR 12 patients, NR or PR to previous line 13 patients), median of previous lines 3 (1-6). The other 5 patients received Bendamustine as frontline therapy. All patients received a 2 days schedule every 28 days; the most frequent Bendamustine dose/day was 90 mg/m2/day dose (80%). Combined treatment with Rituximab occurred in 84% cases. Median number of cycles: 4 (1-8). Response and outcome: A total of 28 patients completed treatment and were eligible for effectiveness assessment. The overall response rate was 56% (CR 23%; uCR 7%; PR 26%). At the time of reporting with a mean follow up of 20. 1 months 19 patients are alive and 7 in CR. All cases of death were progression related. Regarding toxicity, the most common adverse events were hematologic toxicity. Grade 3/4 neutropenia: 10 cases and only 3 episodes of febrile neutropenia. Grade 3/4 anemia: 6 cases. Extra-hematologic toxicity was infrequent and neither dose reduction nor treatment delays, nor hospitalization were influenced by it. No death was attributed to Bendamustine treatment. **Conclusions.** In summary this retrospective study shows that Bendamustine is safe and effective in aggressive lymphoma even in heavily pre-treated patients.

1670

AUTOLOGOUS STEM CELL TRANSPLANT FOR RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN THE RITUXIMAB ERA: A SINGLE INSTITUTION EXPERIENCE

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Background. Rituximab has become an essential key component in the first line treatment of DLBCL in combination with chemotherapy. However, there are limited data in the influence of prior rituximab exposure on the outcome of patients with relapsed/refractoty disease who received a stem cell transplantation as salvage therapy. **Aims.** To compare the outcome of relapsed/refractory DLBCL patients receiving an autologous stem cell transplant as salvage therapy in the rituximab era compared to non-rituximab exposed patients. Patients and **Methods.** We included 85 patients consecutively diagnosed of DLBCL between May 1991 and Nov 2010. They received high dose chemotherapy either conditioned with BEAC (n=) or BEAM (n=). Survival was calculated from date of transplant until death or last follow-up, and time to progression from date of transplant until progression or last follow-up. We employed the cox proportional model for univariate and multivariate analysis and survival curves were built with KM and compared with log rank test.

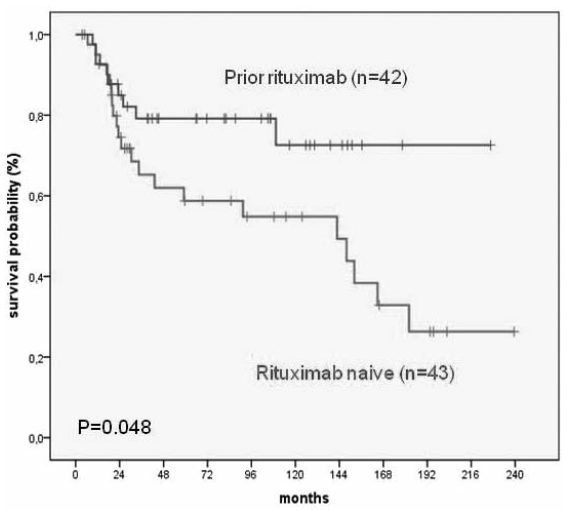

Figure 1.

Results. Median age was 50 years (19 to 66), sex male 46 (54%), histologies DLBCL 68, B rich T cell 9, mediastinal 6, unclassified 2. Stem cell source was: PB 65, bone marrow 6, both 13. Previous treatment: 1: 11 (13%), 2: 49 (58%), ≥3: 24 (29%). Pretrasplant disease response were: CR: 35 patients (41%), PR 29 (34%), non-response 15 (29%). Prior rituximab exposure: none 43 (52%) at any time (dg or salvage) 42 (48%). The main prognostic factors identified in the univariate analysis for both TTP and SRV were, pre-trasplant response (non-resp vs PR vs CR), prior rituximab, and first line treatment shorter than 12 months. In the multivariate analysis for SRV, only a first response duration less than 12 months (HR 9, CI 95% 2,05 to 3,95, p= 0,001)and pre-trasplant response (HR 3,87, CI 95% 1,32 to 11,4, p=0. 04) retained an independent significance. Patients who received rituximab either as part of induction/salvage chemotherapy or just in the salvage schedule had a longer TTP (5-year, 79,2 % vs 58,7%, p=0. 04) and SRV (5-year, 67,5% vs 51,6%, p=0,15) than the rituximab-naïve patients (Figure 1). However patients who received rituximab both at diagnosis and relapse had similar prognosis than the naïve ones. **Conclusions.** Patients with DLBCL exposed to rituximab who received an autologous stem cell transplantation as salvage therapy had a better prognosis in terms of progression and survival than naïve rituximab patients. This benefit seems to be restricted to patients exposed to the monoclonal as part of the salvage therapy.

1671

LOW-DOSE ETOPOSIDE AND CYTARABINE: A PALLIATIVE TREATMENT OPTION IN PATIENTS WITH REFRACTORY/RELAPSED DIFFUSE LARGE B CELL LYMPHOMA NOT ELIGIBLE FOR INTENSIVE SALVAGE CHEMOTHERAPY

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Background. Up to 50% of patients with Diffuse Large B Cell Lymphoma (DLBCL) have refractory/relapsed disease following initial first-line chemotherapy. Prognosis is very poor in patients who fail to respond to or those considered unfit for salvage high-dose therapy with autologous stem cell support. The focus of treatment in these patients is palliation with the aim of improving quality of life. Following demonstration of activity in the salvage treatment of DLBCL in higher doses, a low dose chemotherapy regimen containing oral etoposide and subcutaneous cytarabine has been used at St Bartholomew's since 2008. This replaced the LD-56 chemotherapy regimen which had shown a response rate (RR) of 17% in this patient cohort. **Aims.** The aim of this review was to evaluate the RR and toxicity profile of this regimen in comparison to other low-dose chemotherapy schedules used in this palliative clinical setting. **Methods.** Data was collected on all patients who received this schedule between September 2008 and November 2011. Atotal of 20 patients with relapsed/refractory DLBCL were identified. Oral etoposide (100 mg/m2) and subcutaneous cytarabine (100 mg/m2) were both given on day 1-5 of a 21 day cycle. Treatment was given until disease progression, death, discontinuation due to toxicity or the achievement of remission status. The mean age was 69 years (range 35-81). Twenty percent of patients had an ECOG of >2. Evidence of extranodal disease was seen in 75% of patients and 80% of patients had an elevated LDH >480 IU/L. Patients had received one, two or three or more lines of previous treatment in 40%, 45% and 15% respectively. **Results.** A response was observed in 10 of 20 patients (50%). A partial response (PR) was seen in 7 patients (35%). Two of these 7 patients progressed following an initial response. Three patients (15%) achieved a complete response (CR): one stopped treatment at 5 cycles and 2 stopped following 6 cycles. Two of the patients who achieved CR were re-treated with the same chemotherapy regimen at the point of disease progression: one was treated with a further 4 cycles following a progression free survival (PFS) of 1 year and one was treated with a further 3 cycles following a PFS of 7 months. Only 2 out of the 20 patients (10%) stopped treatment due toxicity (worsened performance status and neutropenic sepsis). The median overall survival (OS) across all patients was 7. 9 months (range 1-39 months). In responders, the median OS was 13. 8 months. Receiving less than 3 lines of prior chemotherapy correlated with an improved overall response rate and overall survival. **Conclusions.** This regimen was effective in patients with refractory/relapsed DLBCL treated with palliative intend. A high response rate of 50% was documented which compares favourably to previously reported data. As an outpatient treatment with limited toxicity this schedule is an attractive palliative chemotherapy regimen for patients in this poor prognostic group. These results further emphasize the need for 'palliative' studies in DLBCL.

1672

BENDAMUSTINE IN PATIENTS WITH RENAL IMPAIRMENT: A RETROSPECTIVE SAFETY ASSESSMENT

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Background. Patients with non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) are at risk for renal impairment as the median age at diagnosis is >65 years. Bendamustine is a bifunctional alkylating agent with efficacy via several pathways and a manageable toxicity profile in NHL and CLL. **Aims.** There is a lack of published research on bendamustine in renally impaired NHL and CLL patients; therefore, a retrospective database analysis was designed to explore bendamustine's safety profile in this population. The study's primary aim was to evaluate laboratory toxicities in the NHL and CLL groups. **Methods.** In this retrospective study of 2 electronic medical record databases from US outpatient oncology practices, patients with NHL and CLL who had received ≥1 dose of bendamustine alone or with rituximab were identified and divided into investigator-defined subgroups: nonrenally impaired (creatinine clearance [CrCL] ≥40 or ≥60 mL/min) and renally impaired (CrCL <40 mL/min). The study collected data on the presence/absence and grade of ≥60 laboratory and physician-reported adverse events (AEs). Patients who received drugs with >5% risk of causing renal damage (eg, cisplatin, cyclosporine) were excluded. **Results.** Of 379 CLL patients, 42 had CrCL <40, 144 had CrCL ≥40-60, and 193 CrCL ≥60 mL/min; of 561 NHL patients, 62 had CrCL <40, 153 had CrCL ≥40-60, and 346 had CrCL ≥60 mL/min. Baseline characteristics were similar, except for more advanced age, shorter mean disease duration, and a slightly lower initial bendamustine dose in renally impaired patients. Mean total bendamustine exposure was 12 weeks for renally impaired vs 15 weeks for nonrenally impaired CLL patients, and ~16 weeks for both NHL groups. No statistically significant differences in laboratory toxicities were seen among the CLL groups, but renally impaired NHL patients had an increased risk for grade 3/4 thrombocytopenia (Cox hazard ratio [HR]: 2. 57; 95% CI: 1. 13, 5. 85) vs those with CrCL ≥60 mL/min. In combined CLL and NHL groups, only grade 3/4 BUN increase was significantly higher for renally impaired patients vs those with CrCL ≥40 mL/min (HR: 2. 36; 95% CI: 1. 15, 4. 86) or ≥60 mL/min (HR: 4. 46; 95% CI: 1. 79, 11. 14). **Summary and Conclusions.** Although sample size and time of exposure limit the power of this study, the results support the safety profile of bendamustine in renally impaired NHL and CLL patients. Increased risk was identified among renally impaired patients for only 2 of the ≥60 evaluated AEs: thrombocytopenia and increased BUN levels. Monitoring blood counts (including platelets) and renal function is probably prudent. Support - Teva Pharmaceutical Industries Ltd.

1673

EFFECTS ON BENDAMUSTINE PHARMACOKINETICS (PK) WHEN ADMINISTERED IN COMBINATION WITH RITUXIMAB IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA (NHL) AND MANTLE CELL LYMPHOMA (MCL)

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Background. The PK profile of bendamustine as a monotherapy in adult patients is well described. Because elimination pathways of small molecules and monoclonal antibodies are distinct, a direct drug interaction between bendamustine and rituximab was not expected. **Aims.** To characterize the effect of rituximab on the PK profile of bendamustine when the 2 are used as combination therapy. **Methods.** In order to confirm the lack of a PK interaction, the population PK model that was developed based on bendamustine monotherapy (120 mg/m^2) was evaluated to determine its applicability to combination therapy (rituximab 375 mg/m^2 on day 1, bendamustine 90 mg/m^2 on days 1 and 2) in patients with NHL and MCL. Once the applicability was confirmed, model-predicted Bayesian bendamustine clearance (CL) values for monotherapy and combination therapy were generated and compared using the Wilcoxon signed rank test on the log-transformed CL values (alpha=0. 05). Patients from the open-label, phase 3 study of bendamustine in combination with rituximab provided informed consent. **Results.** The previously developed population PK model in patients receiving bendamustine monotherapy was used to generate 500 sets of single-agent bendamustine concentrations at the same sampling

times and bendamustine dosing regimens as those observed in the combination-with-rituximab study. Median, 10th, and 90th percentiles of observed bendamustine concentrations following combination therapy and simulated bendamustine concentrations following monotherapy exhibited similar patterns (see Figure 1). Although slightly greater than 10% of the observed data fall above (13%) or below (15%) the specific percentiles, the previously developed monotherapy population PK model was considered applicable to the data following combination therapy. The 25th to 75th percentiles of the bendamustine CL estimates were similar for combination and monotherapy with median values within 8% of each other. The 2-sided Wilcoxon signed rank test of the log-transformed CL values did not show a statistical difference between the 2 groups (P >0. 61). **Summary and Conclusions.** Comparison of bendamustine plasma concentrations and CL estimates in patients receiving bendamustine in combination with rituximab to those in patients receiving bendamustine alone indicate that rituximab does not affect systemic exposure to bendamustine. **Support.** Teva Pharmaceutical Industries Ltd.

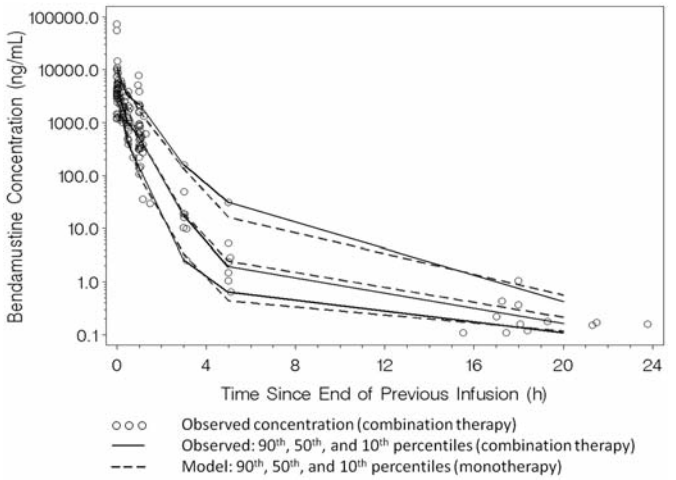

Figure 1. Observed (combination therapy) and model (monotherapy) Bendamustine plasma concentrations.

1674

NEUTROPENIA IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS OF LARGE GRANULAR LYMPHOCYTES: ANALYSIS OF 38 ITALIAN PATIENTS

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Background. Chronic proliferations of large granular lymphocyte (LGL) are rare disorders characterized by expansion of cytotoxic T or NK cells. Relevant clinical featuresinclude neutropenia and anemia, either symptomatic or asymptomatic. It is widely accepted that symptomatic neutropenia requires treatment, however the best approach for asymptomatic neutropenia has not been established. Recently, severe neutropenia has been reported as a criterium for starting therapy. We report data on 38 cases with moderate or severe neutropenia at presentation either asymptomatic or symptomatic. Patients were admitted in different Italian Centers on behalf of GIMEMA group. **Aims.** To evaluate the more appropriate therapeutical approach for patients with LGL and neutropenia. **Methods.** Diagnosis of lymphoproliferative disorder of LGL was made according to published criteria (Semenzato et al, Blood 1997). Accordingly, 37 patients were diagnosed as LGL leukemia and 1 NK chronic lymphoproliferative disorder (NK-CLPD, WHO 2008). Severe neutropenia was defined as an ANC < 500/uL, moderate neutropenia as ANC between 500 and 1. 000/uL. Symptomatic neutropenia was defined as more than 2 febrile episodes/year or as 1 episode requiring hospitalization. Response criteria were defined according to Lamy and Loughran (Blood 2011). **Results.** Fifteen patients (39%; 5 with severe and 10 with moderate neutropenia) did not receive any treatment. The median duration of neutropenia in this group was 65 months (range 9-202 months). No life threatening infections were observed during follow up. Twentythree patients (61%) underwent immunosuppressive therapy: 16 due to neutropenia-related and 7 for neutropenia-unrelated reasons (joint pain, anemia and fatigue). In particular 11/20 (55%) of patients with severe neutropenia and 5/18 (28%) of patients with moderate neutropenia presented recurrent infections. The most common infections were muco-cutaneous (40%) and respiratory infections (23%). First line therapy consisted of methotrexate (MTX) at 7,5 mg/m^2/week in 47% of patients; cyclosporin A (CyA) at 2-3 mg/Kg/die in 43% of patients and cyclophosphamide (CY) at 50-100mg/die in 10% of patients. G-CSF was also used in 34% of patients. The median time to treatment was 11 months (range 0 - 173). Time to response to first line therapy ranged from 1 to 12 months with overall response rate of 69% (8 CR and 6 PR). The duration of response ranged from 5 to 120 months (mean 46 months). 30% of patients treated for neutropenia-related reasons needed a second line or more treatment still reaching CR/PR, whereas 2 patients died for sepsis. **Conclusions.** Neutropenia is one of the most common presenting features in LGL leukemia. According to published series, the most common types of infections seen in our patient group were mucocutaneous and respiratory tract infections. Treatment was restricted to symptomatic patients, regardless ANC. In our series we didn't observe any death for infection in the untreated group, even in the case of severe neutropenia. Accordingly, starting therapy on the basis of ANC may be at risks of overtreatment, exposing the patient to unnecessary long immunosuppressive regimens. A prospective randomized clinical trials comparing the two strategies (treatment on the basis of ANC versus no treatment) is needed to address this issue.

1675

ROLE OF CONSOLIDATIVE AUTOLOGOUS STEM CELL TRANSPLANTATION IN T-CELL LYMPHOMA PATIENTS: A SINGLE INSTITUTION RETROSPECTIVE ANALYSIS

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Background. T-cell lymphomas(TCL) represent a rare and heterogeneous group of malignancies, exhibiting a poor outcome after conventional chemotherapy. **Aims.** To Evaluate role of ASCT in T-cell lymphomas. **Methods.** Our clinical database was retrospectively reviewed; patients with TCL who underwent Autologous Stem Cell Transplant (ASCT) as a front-line or at disease relapse/progression were considered eligible for evaluation. 34 patients (22 males, 12 females; median age 40. 74 years) received a consolidative ASCT after a median number of 2 (range: 1-11) previous lines of therapy. 14 patients had a peripheral T-cell lymphoma, not otherwise specified, 6 an anaplastic large cell lymphoma (ALK-negative in 2 cases),6 a lymphoblastic lymphoma, 3 an angioimmunoblastic lymphoma and2 a cutaneous anaplastic CD30-positive large cell lymphoma. The remaining three cases were a mycosis fungoides, a T/NK nasal type lymphoma and a pleomorphic lymphoma (according toKiel's classification), respectively. 28 patients presented with stage IV disease, with bone marrow involvement in 8 cases and B symptoms in 17 cases. 14 patients were transplanted in complete response (CR),3 in partial response (PR) and 16 with progressive disease (PD). Disease status was unavailable for 1 patient. **Results.** After transplantation, 24 patients achieved a CR, 4 were in PR, 1 had stable disease and 5 progressed. Overall response rate was 82. 36%, with a complete response rate of 70. 59%. 13 patients (38. 24%) could improve their best response after transplantation; a CR was obtained in 10 cases (29. 41%). 13 patients, all in response after ASCT, experienced disease relapse: 2 of them received an allogeneic bone marrow transplantation, and 1 underwent a tandem ASCT-allogeneic transplant. At a median follow-up of 4. 13 (range 0. 92-15. 03) years, 16 patients are still alive,14 in CR and2 in PR. 17 patients died, mainly for disease progression (14 cases). 10-year overall survival from ASCT is 31. 90%, with a median of 4. 92 years. **Conclusions.** ASCT appears effective as consolidation treatment for responding patients, as well as a feasible salvage therapy for those who relapse or progress.

1676

A NEW PROGNOSTIC SCORE FOR AGGRESSIVE B-CELL LYMPHOMA WITH C-MYC TRANSLOCATION INTEGRATING CLINICAL AND GENETIC FEATURES

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Background. C-MYC translocations are observed in 10% of diffuse large B-cell lymphoma (DLBCL) and 30-50% of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (B-UNC/DLBCL/BL). These lymphomas are associated with aggressive disease and dismal prognosis. Within this group attention has been paid to lymphomas with concurrent C-MYC and BCL2 translocations (double hit lymphomas) and triple hit lymphomas with additional breaks involving the bcl-6 gene locus. We have recently shown in mice that C-MYC&TP53 is associated with poor survival and can be abrogated by additional BCL2 alteration (Schuster *et al.,* Blood 2011). **Aims.** We performed a retrospective evaluation of the outcome of 28 patients with DLBCL or B-UNC/DLBCL/BL according to their genetic alterations and therapy (R-CHOP N=10 (36%) patients vs. B-ALL-GMALL containing HD-ARA-C, N=18, (64%) patients).

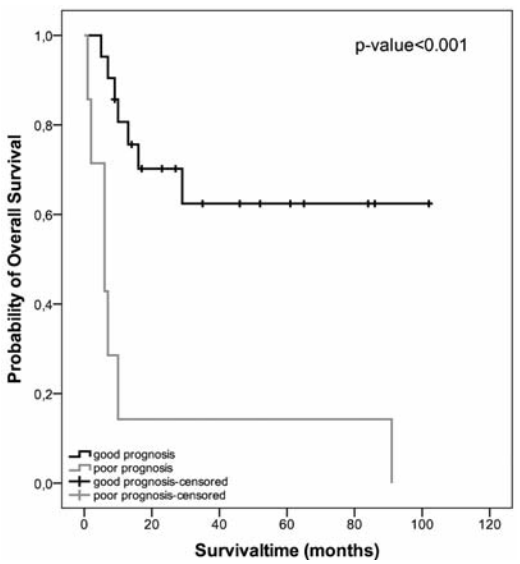

Figure 1. Overall survival of patients according to the new prognostic index.

Methods. C-MYC translocations were screened by FISH. Positive cases were assessed for BCL2 translocations and TP53 deletion by FISH. TP53 mutations were investigated by immunohistochemistry, positive cases were subjected to DNA sequencing. All cases were reviewed by two pathologists, and diagnoses were updated according to the new WHO-classification (2008). We grouped patients according to their genetic alterations in 4 categories (C-MYC only, C-MYC/BCL2, C-MYC/TP53 and triple hit). Statistical analyses were performed using SPSS 17. 0 (SPSS Inc., Chicago, USA) and R for Windows 2. 12. 1 (R Development Core Team) softwares. P-values < 0. 05 were considered as significant. Patient characteristics were tested for potential influence on the survival times using the Cox proportional hazards model in combination with all-possible-subset regression. Based on these results, a new index is suggested allowing for classification of risk. In analogy to international prognostic index (IPI), one point was assigned each to the risk factors 1) positive bone marrow infiltration, 2) high LDH at diagnosis, 3) C-MYC/ BCL2 translocation; two points were assigned to 4) C-MYC/TP53 mutation and no points to 5) presence of only the C-MYC translocation or 6) triple hit aberrations, respectively. The sum was calculated and 0-2 was classified as low versus 3-4 high risk. **Results.** All 28 patients (4 DLBCL, 24 B-UNC/DLBCL/BL) had C-MYC translocation. Nine (32%) patients had only C-MYC translocations, additional BCL2 alterations were found in 9 (32%) B-UNC/DLBCL/BL and TP53 somatic mutations in 6 (21%) patients (2 DLBCL, 4 B-UNC/DLBCL/BL). Four (14%) B-UNC/DLBCL/BL cases showed triple genetic alterations. 1) Overall survival (OS) was significantly different between the four groups (p=0,048). OS at five years was 75% for C-MYC, 71% for triple hit, 35% for C-MYC/BCL2 and 17% for C-MYC/TP53. 2) No significant difference was found between therapeutic regiments indicating no advantage for aggressive chemotherapy. 3) Using the novel prognostic score two groups with good and poor prognosis could be defined (Figure 1). **Conclusions.** Our data indicate that (i) simultaneous C-MYC and TP53 alterations confer high risk. This risk is considerably ameliorated when an additional BCL-2 aberration is present. (ii) GMALL-ALL protocol is non-superior to R-CHOP. (iii) The prognostic score integrating clinical and genetic features is able to predict outcome and should be evaluated in larger studies.

1677

NODAL PERIPHERAL T-CELL LYMPHOMA (PTCL): CHARACTERISTICS, PROGNOSIS AND OUTCOME OF 120 PATIENTS

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Background. Peripheral T-cell Non Hodgkin lymphomas (PTCL) are rare diseases in the Western World. Among them, the most common entities are the nodal PTCL. Due to their rarity and difficulties in their diagnosis and classification, reports from large series are scarce in the literature. **Aims.** To record and analyze nodal PTCL patients' characteristics, outcome and prognostic factors. **Methods.** We retrospectively studied 120 consecutive patients with nodal PTCL diagnosed and treated in 2 University Hematology Units between1991-2011.

Table 1. Nodal PTCL patients' characteristics.

Patient characteristics	ALCL (%)	AITL (%)	PTCL-NOS (%)	p
Age>60	22	50	47	0.02
Male gender	71	49	71	0.07
Splenomegaly	7	49	19	<0.001
Bone marrow involvement	5	47	27	<0.001
B-symptoms	39	65	53	0.07
Stage III-IV	38	84	75	<0.001
PS≥2	10	35	12	0.01
E-sites≥2	13	42	31	0.02
Increased LDH	44	63	59	0.21
IPI				
Low	53	14	22	0.006
Low-intermediate	26	21	26	
High-intermediate	16	36	37	
High	5	29	15	
PIT (# factors)				
0	39	7	26	
1	44	25	26	0.001
2	15	32	33	
3-4	3	36	15	

Results. Median age at diagnosis was 57 years (13-91) with 40% being >60 years, 63% were male, 65% had clinical stage III-IV, 53% B-symptoms,19% PS≥2, 54% abnormal LDH, 54% extranodal disease (27% in ≥2 sites), 26%

bone marrow and 25% splenic involvement. Histology was as follows: 34% anaplastic large cell lymphoma (ALCL, 11% ALK+, 13% ALK-, 10% unknown), 31% angioimmunoblastic lymphoma (AITL) and 32% peripheral T-cell not otherwise specified lymphoma (PTCL-NOS). Table 1 presents patients' characteristics. Initial treatment was CHOP/CHOEP/ CNOP in 81%. At a median follow-up of 51 months the 5-year progression free survival (PFS) and overall survival (OS) were 39% and 54% respectively. 65 events of relapse/progression were recorded: 10 proceeded to autologous stem cell transplantation with 8/10 being cured, whereas 6/65 achieved long-term remission following 2nd line conventional chemotherapy. Only 5/65 patients who progressed or relapsed, did so after 2 years from diagnosis. Univariate analysis revealed that advanced stage (p=0. 02), elevated LDH (p=0. 02), ≥2 extranodal sites (p=0. 004) and non-anaplastic histology (p=0. 02) were statistically significant adverse prognostic factors for PFS. The aforementioned factors in addition to age>60 correlated with an adverse OS, as well (p=0. 04, 0. 02, 0. 045, 0. 02 and 0. 0004 respectively). Although both IPI and PIT had prognostic significance for PFS (p=0. 02 and 0. 03) and OS (both p=0. 02), they were particularly effective in separating the good prognosis patients but not those with a worse outcome. Multivariate analysis indicated that involvement of ≥2 extranodal sites (p=0. 02) was the sole significant prognostic factor for PFS, whereas age>60 years(p=0. 003) was the only significant parameter for OS. In addition ≥2 extranodal sites, and high LDH proved of marginal independent prognostic significance for OS(p=0. 06 and 0. 08 respectively). **Conclusions.** Nodal peripheral T- cell lymphomas are associated with poor prognosis. ALCL and AITL are characterized by distinct clinical and biological features. Disease can be successfully controlled in 40% of the patients with a low probability of relapse after the first two years. Extranodal involvement in ≥2 sites is a strong prognostic indicator, whereas IPI and PIT have a limited prognostic value.

1678

EXTRANODAL NK/T-CELL LYMPHOMA ASSOCIATED WITH REACTIVE HEMOPHAGOCYTIC SYNDROME:A RETROSPECTIVE STUDY

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Background. Extranodal NK/T-cell lymphoma associated hemophagocytic syndrome (NK/T-LAHS) is a rare life-threatening disorder, which has been mainly reported inAsia countries. It progresses rapidly, and the prognosis is usually poor. However, the pathogenesis and prognostic factors of NK/T-LAHS are not fully understood. **Aims.** To find out more about the disease, we conducted a retrospective study on the NK/T-LAHS patients diagnosed and treated by our hospital.

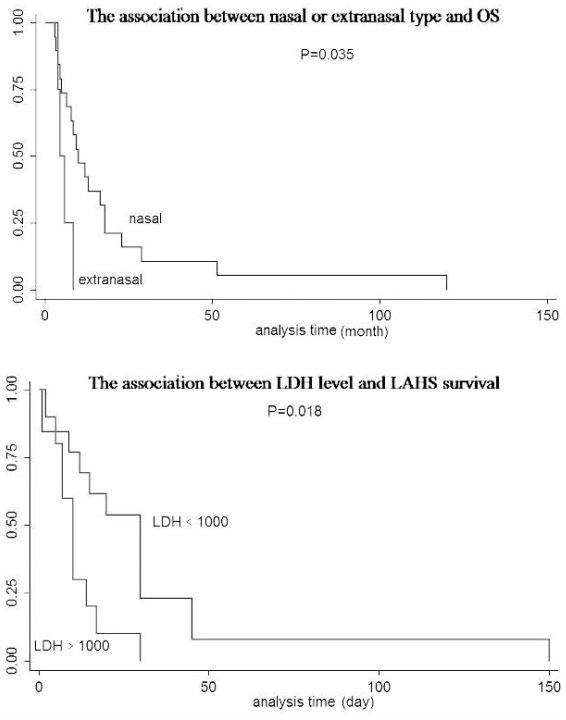

Figure 1. Survival rates analysis for NK/T-LAHS.

Methods. 23 NK/T- LAHS patients diagnosed between July 2006 and September2011 in Fudan University Shanghai Cancer Center were included. The information of clinical features, laboratory findings, treatment and prognosis of these patients were collected and analyzed. **Results.** Of the 23 NK/T- LAHS patients, the median age was 45 years old and 15(65. 2%) were male. 11(47. 8%) had an Ann Arbor stage of Ⅲ/Ⅳ; 19(82. 6%) cases originated from nasal cavity, nasal sinuses, and tonsil, while the other 4 (17. 4%) cases originated from colon, cervix, or skin. LDH levels were elevated in all 23 cases at the time of diagnosis, and 10(43. 5%) cases showed elevated LDH levels of more than 1000IU/L at the onset of HPS, which predicted a poor prognosis. NK/T-LAHS was characterized by fever, pancytopenia, liver dysfunction, and hypertriglyceridemia, but only 9 cases (39. 1%) were found hemophagocytosis in bone marrow. The median OS was 8. 5 months from the diagnosis of NK/T cell lymphoma, and the median LAHS survival time was 14 days. Chemotherapy and high-dose *glucocorticoids were not so effect to* NK/T-LAHS that almost 100% cases died soon. **Conclusions.** NK/T-LAHS usually occurs in the nasal type of NK/T cell lymphoma, but the prognosis of extranasal type is poorer. The rate of its definite diagnosis at the early stage is low. Most of the patients were died of multiorgan failure, infection and bleeding. The original lesion and LDH level may be prognostic factors. There was no effect treatment for NK/T-LAHS that it remains a challenge.

1679

SPLENIC MARGINAL ZONE LYMPHOMA FLOWS WITH AUTOIMMUNE HEMOLYTIC ANEMIA

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Background. Splenic marginal zone lymphoma (SMZL) - B-cell indolent lymphoma which characterized by splenomegaly, bone marrow involvement and with immunophenotype of B-lymphocytes to the marginal zone of secondary follicles. It has a variety of clinical manifestations, immunological and cytogenetic features and often occurs with autoimmune hemolytic anemia (AIHA). Aims. The aim of our study was to determine the frequency and approaches to treatment of SMZL flows with AIHA. **Materials and Methods.** From January 2000 to February2012 intheHematologicalResearchCenter(Moscow, RF), observed 122 patients with SMZL, of which 8 (6. 6%) patients the disease proceeded with AIHA. The diagnosis of SMZL was established on the basis of clinical and immunophenotypic data. All patients were female; mean age was 63. 7 years (46-78 years). In all patients, the disease debuted with splenomegaly, bone marrow involvement and a clinical picture of hemolytic anemia: a sudden jaundice, increased levels of total bilirubin by free fraction, anemia, reticulocytosis and positive Coombs test. There was decrease of hemoglobin of 36 g/l to 94 g/l in peripheral blood, which was accompanied by a low increase in the number of reticulocytes. Increased LDH was observed in all cases and could indicate how the activity of the tumor and the intensity of hemolysis. Coombs test in a small titer was positive in all cases. M-component were found in all cases. Splenectomy was performed in all patients as first-line therapy. **Results.** In all patients after splenectomy observed clinical and hematological improvement, which manifested no signs of hemolysis (red blood cell count the hemoglobin level returned to normal, absence of reticulocytosis, the normalization of the concentration of LDH, negative Coombs test and the disappearance of M-component). Median duration of response was 58 months (from 38-90 months). In two patients after splenectomy in 41 and 43 months. respectively, were marked by signs of tumor progression and recurrence of hemolysis, which was the reason for the appointment of a standard dose of Rituximab and obtained a complete remission with disappearance of signs of hemolysis and bone marrow lesions. Duration of remission was 24 and 32 months respectively. **Conclusions.** SMZL often occurs with AIHA and characterized by a low increase in the number of reticulocytes, a good response to splenectomy. In the case of progression and/or renewal of hemolysis there was effective use of Rituximab.

1680

LARGE-CELL TRANSFORMATION OF SPLENIC MARGINAL ZONE LYMPHOMA

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Background. Splenic marginal zone lymphoma (SMZL) - indolent B-cell lymphoma, which is characterized with splenomegaly, bone marrow involvement and favorable course. **Aims.** The aim of our work was to study the frequency of occur-

rence of large-cell transformation in patients with SMZL, the course and treatment approaches. **Materials and Methods.** From January 2000 to February2012 inthe Hematology Research Center (Moscow, Russia), observed 122 patients with SMZL, of which 11 (9%) patients had signs of large-cell transformation. The diagnosis of SMZL was established on the basis of clinical and immunophenotypic data. Large-cell transformation was diagnosed by histological examination of the spleen removed (8 patients) and an average of 21. 7 months (3-51 months) after splenectomy at the time of progression for histological examination of peripheral lymph nodes. The average age was 56. 7 years (33-77 years). All patients had stage IV disease and B symptoms. Anemia was observed in 9 patients, leukopenia in 5 patients, leukocytosis in 3 patients, thrombocytopenia in 9 patients. Increased LDH was observed in all cases and the M-component in 6 patients. **Results.** Histological examination of bone marrow revealed focal small and medium-sized lymphoid cells infiltration of bone marrow, which was accompanied by a decrease in the number of normal hematopoietic cells. No signs of large-cell transformation (the presence of layers of large cells, abundant figures of mitoses in bone marrow) were found. In 8 patients larg-cell transformation were diagnosed at the time of splenectomy. Histological examination of spleen revealed blurring the picture of the structure of spleen, remained nodular growths. In the cellular layers of the composition was dominated by large lymphoid cells with rejuvenated chromatin structure of the nucleus and nucleoli expressed polymorphism. After splenectomy after an average of 21. 7 months (3-51 months) in three patients was developed disease progression with manifestation with massive lymphadenopathy. Histological examination of biopsies of lymph nodes revealed diffuse infiltration of large cells, abundant mitotic figures. According to the immunophenotypic study was observed of expression of activation markers (CD71, CD38). In most cases there was high proliferative activity (12-20%). All patients with large-cell transformation received chemotherapy CHOP-21 (4 patients with the addition of Rituximab). Complete remission duration of 36. 3 months on average (20-70 months) obtained in 7 (63. 6%) patients, partial response - in 2 patients. Two patients died of progressive disease and infectious complications. **Conclusions.** The large-cell transformation of SMZL can be diagnosed as at the time of splenectomy and after splenectomy in tumor progression. Chemotherapy CHOP-21 leads to complete remission in 63. 6% of patients for up to 3 years.

1681

TOXICITY PROFILES OF TWO PLATINUM-BASED SALVAGE REGIMENS, DHAP VERSUS ESHAP, IN RELAPSED/REFRACTORY LYMPHOMA

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Background. Platinum- or ifosfamide-based salvage therapies, such as ESHAP, ICE, and DHAP, are frequently used regimens in relapsed/refractory lymphomas. Few prospective studies demonstrated no superiority of these protocols against to each other. While DHAP and ICE can be applied in outpatient setting, ESHAP is administered at medical ward. The most important adverse effect of salvage therapies is hematologic toxicity. However, renal toxicity is an other important problem observed with platinum-based regimens. **Aims.** The aim of the study was to compare the hematologic and non-hematological toxicity profiles of two different platinum-based salvage chemotherapy regimens used in relapsed/refractory lymphoma. **Methods.** We evaluated 51 patients with HL and NHL who were treated with DHAP and ESHAP regimens (n=33 for ESHAP and n=18 for DHAP) between January 2000 and July 2010. These patients had received a total of 153 cycles (91 ESHAP and 62 DHAP). Data were retrospectively collected from patients' chart records and electronic patient inventory. **Results.** There were no significant differences regarding serious hematologic toxicities (grade 3-4) between two salvage regimens. Platelet and red blood cell transfusions were given to 28 (55%) and 27 (53%) patients, respectively. Febrile neutropenia occurred in 11 (21. 5%) patients. Treatment-related death was not observed. Renal toxicity, platelet transfusion requirement, febrile neutropenia, and treatment delay due to treatment toxicity were observed more frequently in DHAP group in univariate analysis. Receiving DHAP regimen was found to be an independent risk factor for renal toxicity (OR=23. 6, p=0. 03) in multivariate analysis. However, the number of platelet transfusions, febrile neutropenia, and treatment delay due to treatment toxicity has not reached statistical significance in the latter analysis. Time-to-next cycle interval following the first salvage cycle were longer in patients receiving ESHAP (median 35 days vs 27 days, p=0. 008). Overall response remained significantly higher in DHAP group in multivariate analysis (87% vs 48%, p=0. 04). There was no significant difference between two groups (ESHAP and DHAP) in terms of median survival. **Summary and Conclusions.** DHAP regimen is associated with higher response rates but has no survival advantage. Since it can be administered in outpatient setting, superior efficacy of DHAP can be partly explained by its use with regular intervals. Outpatient DHAP regimen may be preferred in clinics where inpatient facility is not capable to supply chemotherapy in regular intervals. Although the hematologic and non-hematologic toxicity profiles were similar, increased risk for renal toxicity should be considered for patients planned to receive DHAP regimen.

1682

CLINICAL FEATURES OF PRIMARY GASTROINTESTINAL TRACK NATURAL KILLER/T CELL LYMPHOMA

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Background. Extranodal natural killer/T cell lymphoma, especially those occurring in the GI tract is extremely rare. There were only few case reports, but no papers of investigation clinical features, analysis prognostic factor and description about optimal treatment strategies. **Aims.** We aimed to indentify clinical features of GI NK/T cell lymphoma and analysis treatment outcome. **Methods.** Between 2005 and 2011, 23 patients were diagnosed of GI NK/T cell lymphoma. We collected clinical finding including treatment outcome retrospectively. **Results.** The median age was 49 (range 17-72) and 69. 6% was male. Initial symptom or sign were abdominal pain (43. 5%), nasal obstruction symptom (30. 4%), hematochezia (17. 4%) and bowel perforation (13. 0%). 87% of patients were Ann Arbor stage III/IV AND 30. 4% patients has nasal cavity involvement. Each group for IPI score, 26. 1% for low, 30. 4% for low-intermediate, 34. 8% for high-intermediate and 8. 7% for high IPI score. However, 30. 4% patients had KPI score 2 and 56. 5% patients had KPI score 3 or 4. Most common involved site of GI was small bowel (ileum or jejunum). 19(82. 6%) patients died and median overall survival and progression free survival was 6. 2 months and 2. 3 months. First regimen was CHOP for 12 patients, SMILE for 5 patients and VIPD for 3 patients. 11 patients received 2nd line chemotherapy. Among chemotherapy, there was no difference of OS (5. 6months vs 9. 3 months vs 8. 1 months; *P*-value 0. 801) and PFS (3. 0months vs 2. 0months vs 3. 9months; *P*-value 0. 725). **Conclusions.** Primary GI NK/T cell lymphoma is highly aggressive malignancy which has poor OS and PFS.

1683

PREVENTION OF CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING WITH PALONOSETRON IN PATIENTS WITH NON-HODGKIN'S LYMPHOMAS UNDERGOING REPEATED CYCLES OF MODERATELY EMETOGENIC CHEMOTHERAPY

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Background. Patients undergoing chemotherapy (CT) commonly receive repeated cycles of treatment. While chemotherapy-induced nausea and vomiting (CINV) is known to be a mostly feared side effect affecting patient qualityof life and leading to possible chemotherapy discontinuation. Associated increased health care resources and prevention of CINV in cycle 1 diminishes its potential in subsequent cycles. Therefore, optimizing antiemetic prophylaxis at initiation of CT is critical. Palonosetron (PALO), a potent 5-HT_3 receptor antagonist (RA) with a distinctly different pharmacokinetic and receptor binding profile, has demonstrated improved CINV protection compared to older 5-HT_3 RAs in multiple phase 3 and 4 single-CT-cycle clinical trials; however, few studies have evaluated PALO over multi-cycles of CT. **Aims.** This was a prospective, multicenter, single-arm study designed to assess the efficacy and safety of single IV doses of PALO 0. 25 mg in preventing CINV in chemotherapy-naïve patients with Non-Hodgkin's Lymphomas (NHL) scheduled to receive at least 2 and up to 4 repeated, consecutive cycles of single day moderately emetogenic chemotherapy (CHOP, R-CHOP or ProMACE-CytaBOM). **Methods.** Corticosteroids (prednisone) is one of the components of these regimens and were not added for antiemetic purposes. The primary efficacy endpoint was complete response (CR: defined as no emetic episode and no use of rescue medication) during the overall phase (0-120 hrs) following CT during each cycle. Secondary endpoints included CR during the acute and delayed phases (0-24h, 24-120 h respectively), and the evaluation of emesis-free patients' rate and nausea severity (according to a 100 mm VAS) during all 3 time intervals. The safety profile and adverse events were also assessed. Informed consent was obtained from all patients. **Results.** A total of 88 patients with either B-cell or T-cell NHL (91% and 9% respectively) received PALO during Total Study Cycles (TSC: 317; mean 3. 6; median 4). The majority of patients were males (60%) with a mean age of 59. 7 yrs who received either CHOP (47%) or R-CHOP (52%). Primary endpoint, CR overall rate reached 76. 7% in TSC. A

high rate of study patients, always > 86%, remained emesis free during each cycle, reaching 90. 5% emesis free patients in the overall phase of TSC. Complete protection, defined as no vomiting no rescue therapy and no more than mild nausea was achieved in the 72. 2% of all patients in the overall phase of TSC, and the 68. 8% of patients was nausea-free in the overall phase of TSC. A very low impact of nausea and vomiting on daily life activities was evidenced by using a modified FLIE questionnaire (121 out of 126 total score where 126 indicates no impact on daily life). PALO was well tolerated and showed a high safety profile over repeated cycles, with few adverse events possibly/probably/ definitely related to study drug, with a low incidence of the most frequent, i.e. headache and constipation (1% and 2% respectively) . **Conclusions.** PALO administered as a single fixed IV dose before chemotherapy has been showed to be very effective and safe on CINV prophylaxis in patients with NHL undergoing repeated cycles of moderately emetogenic chemotherapy.

1684

PROGNOSTIC FACTORS AND OUTCOMES IN PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF ADRENAL GLAND TREATED WITH RITUXIMAB-CHOP CHEMOTHERAPY

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Background. Primary adrenal non-Hodgkin's lymphoma is extremely rare, prognosis was known to be very poor. **Aims.** The objective of this study was to define clinical features and treatment outcomes of primary adrenal diffuse large B cell lymphoma (DLBCL).

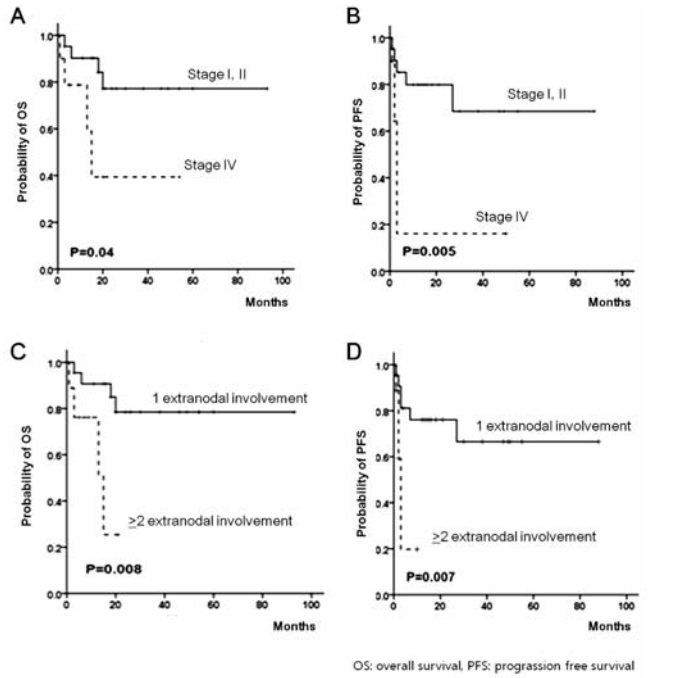

Figure 1. OS (A) and PFS (B) according to new staging system and OS (C) and PFS (D) according to modified number of extranodal involvement sites.

Methods. A retrospective study of 31 patients diagnosed with primary adrenal DLBCL treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) between 1998 and 2010 at 13 Korean institutions were analyzed in this study. **Results.** Median age was 64 years with male to female ratio 2. 8:1. Most common presenting symptom was an abdominal pain (41. 9%) with 53. 5 % of patients presenting with bilateral adrenal gland involvement. Lactate dehydrogenase was elevated in 24 of 28 (85. 7%) patients. Complete remission was 58. 1% and overall response rate was 87. 0% after R-CHOP chemotherapy. Two of relapsed four patients were occurred at central nerve system (CNS). The 2-year estimate for overall survival (OS) was 68. 3% and progression free survival was 58. 0%. There was no survival difference according to Ann Arbor staging, IPI scoring system and bilateral adrenal gland involvement. We defined new staging system and modified number of extranodal involvement regarding bilateral involvement as on extranodal site. Those factors were associated with OS, respectively (p=0. 04, p=0. 008) (Figure 1). **Conclusion.** This study suggested that treatment outcomes of primary adrenal DLBCL treated with R-CHOP was not poor contrary to previous report. New staging system and modified number of extranodal involvement could be a reasonable options to predict R-CHOP response. CNS prophylaxis added to initial treatment might be considered although absolute number of CNS relapse is not high.

1685

LUPUS ANTICOAGULANT, VENOUS THROMBOSIS AND OTHER AUTOIMMUNE PHENOMENA IN PATIENTS WITH SPLENIC MARGINAL ZONE LYMPHOMA: Results FROM THE VIENNA COOPERATIVE STUDY

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Background. Splenic Marginal Zone Lymphoma (SMZL) is a low malignant lymphoma, which may be associated with immune-mediated paraneoplastic conditions. The association of the lupus anticoagulant (caused by a phospholipid antibody) with SMZL has been reported in a number of cases, but no systematic data on the prevalence of lupus anticoagulant (LA) in this lymphoma have been obtained. **Aims.** The aim of our study was to investigate the prevalence of LA in a large group of histologically well-defined SMZL. **Methods.** This was a retrospective cooperative study of four haematological centres in Vienna. Patients still alive at time of the data analysis were asked to visit the outpatient services of the respective centres. Blood was taken for coagulation studies (in particular lupus anticoagulant) and for other potential immune mediated conditions. Additionally, clinical haematological and coagulation laboratory data were obtained from archival material for all patients, including those who have died or did not attend the follow up examination. The study was approved by the ethics committees of all institutions. In case of prolongation of the Activated Partial Thromboplastin time (APTT-STA, Diagnostica Stago) appropriate clotting factors or activities were determined. If they were normal APTT was determined with a lupus sensitive reagent (PTT-LA, Diagnostica Stago) and if it was prolonged the ability of normal plasma to normalize the prolonged APTT was tested. If APTT was normalized a confirmation test according to ISTH recommendation was done (definite LA). **Results.** The study included 70 patients. 22 were untreated, 22 had undergone splenectomy alone and 26 chemotherapy (12 with and 14 without splenectomy). The median age of all patients examined for LA was 68 (range 32-91) years, and the male/female ratio was 44/26. Nine patients (12. 9%) had a definite LA. The median age of LA patients was 57 (range 32-74) years and the male/female ratio was 4/5. Three out of 9 LA patients (33. 3%) and 6 out of 61 non-LA patients (9. 8%) had a documented venous thromboembolism. Other immune mediated conditions in patients with LA were autoimmune hemolytic anemia in 4, and C1 esterase inhibitor deficiency in one case. **Conclusions.** The prevalence of LA is high in SMZL. LA in this lymphoma is associated with venous thromboembolism and other immune mediated conditions.

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COMBINATION OF RITUXIMAB-CHLORAMBUCIL AS 1ST LINE TREATMENT FOR GASTRIC MALT LYMPHOMA

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Background. The mucosa associated-lymphoid tissue (MALT) lymphoma of the stomach is the most common extranodal marginal zone lymphoma. It is usually related with helicobacter pylori infection. The optimal treatment is still not defined in patients who do not present h. pylori infection or experience persisting or recurrent disease, after appropriate antihelicobacter medication. **Aims.** The study of characteristics results and follow up of patients with gastric MALT lymphoma who were treated with the rituximab-chlorambucil combination as 1st line chemotherapy regimen. **Patients and Methods.** Between 2002-2011 patients with MALT lymphoma who did not present helicobacter pylori infection or experienced a relapse or persisting MALT lymphoma after antihelicobacter medication were treated with the rituximab-chlorambucil combination. Rituximab (375mg/m2) was administered every 1st day of a 28day cycle and chlorambucil (10mg/d) for 10 days (2nd-12th day). This combination was repeated for 8 cycles and then patients received another 4 cycles with chlorambucil alone (10mg/d for 10 days/month). Their follow up was performed after 4th, 8th and 12th cycle and was consisted of gastroscopy as well as CT scan and bone marrow biopsy, when necessary. Only patients who have completed their 12 month treatment schedule are included in this analysis. **Results.** 31 patients were studied. Median age is 55 years and male to female ratio is 1:1. At diagnosis, 22 had a stage IEA disease, 5 stage II and 4 stage IVA disease with bone marrow infiltration. H. pylori infection was not documented in 6 patients. From the 25 patients, who received antihelicobacter medication on diagnosis, 5 required alternative antibiotic regimens, due to h. pylori resistance to standard treatment . In 22 patients (71%), complete remission (CR) was documented on 4th cycle (8 pts), 8th cycle (7) and 12th cycle (7). All patients with stage II and IV and 13/22 of stage I achieved CR. Partial remission (PR) was achieved in 5 patients, minimal residual disease (MRD) in 3 and stable disease in 1 patient. Five patients presented clonal IgVH rearrangement in the blood; 3/5 achieved only PR at the end of chemotherapy. Median follow up is 38 months. In 21/22 patients the disease remains in CR, while in one patient a transformation to diffuse large B cell lymphoma was observed, 3 months after the end of chemotherapy. In those who achieved a PR, a course with weekly rituximab monotherapy was performed (6 weekly rituximab infusions) and in all but one, a CR was documented. From those who presented MRD, 1 achieved CR 6 months after the end of chemotherapy and 2 remain in MRD. No complications were observed and all patients continued their daily activities throughout treatment. **Conclusions.** The rituximab-chlorambucil combination constitutes a safe and effective option in patients with gastric MALT lymphoma who require chemotherapy. The readministration of rituximab can achieve better results in cases of PR. The design of treatment schedule, as administered in our patients, provides a complication-free course with a satisfactory life quality, throughout therapy.

1687

HIGH-DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR RELAPSED FOLLICULAR LYMPHOMA: THE ARGENTINEAN EXPERIENCE

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Background. since the introduction of rituximab in the treatment of Follicular Lymphoma (FL) the place of High Dose Therapy (HDT) and Autologous Stem Cell Transplantation (ASCT) as salvage therapy in the relapsed setting has been questioned. **Aims.** our objectives were to evaluate the long-term event-free survival (EFS) and overall survival (OS) rates of patients with relapsed or refractory FL who received HDT and ASCT as salvage therapy. **Methods.** we conducted a retrospective analysis of 44 consecutive patients with FL who received HDT and ASCT as salvage therapy in four Argentineans transplant centers from 1995 to 2011. **Results.** 44 patients were transplanted, median age 53 years (range 31-73); 32 (72. 7%) have received rituximab as part of their induction treatment previous ASCT. The conditioning regimens used were CBV 20 (45. 4%), BEAM 19 (43. 2%) and BEAC 5 (11. 4%). No conditioning regimen including TBI was used. The median time from diagnosis of FL to ASCT was 31 months (range 1-150). With a median follow-up of 31 months the median EFS was reached at 29 months and a plateau on the EFS curve was evident starting at 8 years post-ASCT. The median OS was not reached during follow-up, with 5 years projected OS of 63%. Median EFS for patients who received Rituximab as part of their induction treatment previous ASCT versus those who did not was 24 and 47 months respectively [HR 0. 59; IC (0. 25-1. 38); p=0. 24]. EFS rates were not significantly different in patients who received CBV, BEAM and BEAC as conditioning regimens. There were two cases of secondary malignancies (two urotelial carcinomas, one patient received CBV as conditioning regimen and the other BEAM); there were no reports of secondary MDS/AML. **Conclusions.** In this study 50% of patients were free from progression after 29 months of ASCT; HDT follow by ASCT can achieve long-term EFS in patients with relapsed or refractory FL.

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TREATMENT OF PERIPHERAL T-CELL LYMPHOMA WITH AN INTENSIVE PROTOCOL ACEP (ADRIAMYCIN,CYCLOPHOSFAMIDE,ETOPOSIDE AND PREDNISOLONE) AND IFOSFAMIDE SHOWING IMPORTANT RESPONSE AND OVERALL SURVIVAL RATES

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Background. peripheral T-Cell Lymphoma is a group of lymphoid malignancies which has not the chance to be treated confidently as what happened in its counterpart B-Cell Lymphomas. And despite the studies which were retrospective in majority,treatment results still disappointed taking into consideration the aggressive clinical course of disease,so survival is not exceeding 2 years in median. **Aims.** to assessment response rate and overall survival at 5 years using a new intensive combination chemotherapy . **Methods.** patients were enrolled are diagnosed with PTCL confirmed by a reference pathologist treated with the new chemotherapy (ACEP)X 6 and Ifosfamide X 4 at Al-Bairouni university hospital and the study was approved by the Syrian Association of Clinical Oncology. **Results.** 25 patients underwent the treatment most of them have shown a complete response after the completion of the first six cycles (17/25) forming 68% of patients while another 5 patients became complete responders after the completion of treatment to reach to 22 patients still living at 5 years and an overall survival rate of 88%. **Conclusions.** (ACEP) and Ifosfamide seems to be a good choice in PTCLs in the light of good response and overall survival rates taking into account the acceptable toxicity profile but we still need a larger sample to make it an acceptable new combination chemotherapy for PTCLs patients.

1689

SAFETY AND EFFICACY OF RADIOIMMUNOTHERAPY WITH 90Y-RITUXIMAB IN PATIENTS WITH RELAPSED OR REFRACTORY CD20+ B CELL LYMPHOMA: A PILOT STUDY

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Background. Both anti-CD20 antibodies (ibritumomab; Zevalin® and tositumomab; Bexxar®) currently used for radioimmunotherapy (RIT) of relapsed B cell non Hodgkin's lymphoma's are murine immunoglobulins. The use of chimeric antibodies such as rituximab for RIT may potentially increase immune-based anti-tumour activity, improve pharmacokinetics, and reduce immunogenicity (and hence facilitate repeated administrations). **Aims.** The aim of this pilot single arm phase I-II study is to evaluate the safety and efficacy of RIT with 90Yttrium-rituximab. **Methods.** This prospective single-centre study included patients with CD20+ B-cell lymphoma in partial remission or progressive disease after at least 1 line of therapy. The 90Y-rituximab was administered according to a similar schedule as currently approved by the European Medicines Agency for the treatment with 90Y-ibritumomab tiuxetan (Zevalin®). Each patient received:1) Dosimetry with 89Zirconium-rituximab-

PET/CT: i. v. -administration of rituximab at 250 mg/m² followed by the injection of the ^{89}Zr-labelled rituximab (3 mCi), with immuno-PET/CT performed 1h, 3 days and 6 days after tracer injection. 2) Therapeutic phase: one week later, the same infusion of cold rituximab followed by the injection of ^{90}Y-labelled rituximab (0. 3 mCi/kg if platelet count: 100000-150000/mm^3 and 0. 4 mCi/kg if platelet count: > 150000/mm^3). A baseline ^{18}FDG-PET/CT was performed before treatment and response assessment with ^{18}FDG-PET/CT was performed 3 months after the treatment and/or when progressive disease was clinically suspected. All patients signed a written informed consent. **Results.** 27 patients were included with a median age of 61 (range 29-73) years and a median of 2 (1-7) prior therapy regimens. Disease histologies included follicular lymphoma (n=14), predominant nodular lymphocytic CD20+ Hodgkin's lymphoma (n=3), marginal zone lymphoma (n=3), transformed follicular lymphoma (n=2), mantle cell lymphoma (n=2), diffuse large B-cell lymphoma (n=2) and small lymphocytic lymphoma (n=1). The overall response rate (ORR) was 85% (95% CI: 67%-94%), including 17 patients (63%) with complete metabolic response (CR) and 6 patients (22%) with partial responses (PR). Two patients had a stable disease and 2 patients a progressive disease. After a median follow-up of 103 weeks, the Kaplan-Meier estimated median progression-free survival was 51 weeks. Among follicular lymphoma's, the ORR was 100%, including 79% of CR and 21% of PR. Toxicities related to ^{90}Y-rituximab were primarily haematological with spontaneous recovery in all but one patient that needed autologous stem cell transplant for refractory thrombopenia. The incidence of grade 3-4 neutropenia, thrombopenia and anemia were 34%, 41%, and 7% respectively. Nadir blood counts occurred at 4 to 5 weeks following therapy for platelets and at 6 and 8 weeks for white and red cells respectively. Prior treatment with radiotherapy or autologous stem cell transplant as well as bone marrow involvement were not correlated with higher toxicities. Among the relevant long-term side effects, one patient previously treated with R-CHOP chemotherapy and autologous stem cell transplant developed secondary myelodysplasia 2 years after the RIT. **Conclusions.** The results of this study suggest that radioimmunotherapy with ^{90}Y-rituximab in patients with relapsed CD20+ B-cell lymphoma is safe, well tolerated and effective when the Zevalin® treatment schedule is used.

1690

NON-HODGKIN LYMPHOMA IN JORDAN: A RETROSPECTIVE ANALYSIS OF 852 CASES IN A TERTIARY CANCER CENTRE

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Background. Lymphoma is the fourth most common newly diagnosed cancer inJordan, a small country with a population of 5. 85 million. The contribution Non- Hodgkin lymphoma (NHL) to the overall lymphoma burden inJordan, andthe relative frequencies of its major subtypes, have not been hitherto well characterized. OBJECTIVE: to characterize the clinico-pathological features of Non-Hodgkin lymphoma, and the relative contribution to the overall lymphoma burden, in patients referred to King Hussein Cancer Center (KHCC), the major cancer tertiary referral centre inJordan. **Patients and Methods.** A retrospective analysis was conducted of adults (>=18 years) lymphoma patients referred to KHCC, between1/1/2003and31/12/2010. Clinical features andhistological subtypes were prospectively established for all patients registered in the Lymphoma Service Database. Pathology review and submission of original paraffin blocks were mandated for all patients. **Results.** Over the 8 year period of 2003-2010,1329 lymphoma patients were referred to KHCC and registered in the Lymphoma Service Database, of whom 852 (64. 1%) were diagnosed with Non-Hodgkin lymphoma. Among this group all 852 patients were adults 18 years or older (100%), as children are treated in a different department. B-cell lymphomas formed (744) 87. 3% of the NHLs,whereas T-cell lymphomas formed (108)12. 7% of the total. Diffuselarge B-cell lymphoma was the most common subtype 432 (50. 7% ofall NHLs). Follicular centre-cell lymphomas, B-cell smalllymphocytic lymphoma, mantle-cell lymphoma, marginalzone B-cell lymphomas (including MALT lymphomas), and Burkitt lymphomaamounted to 66 (7. 7%),57(6. 6%), 12(1. 4%), and 24 (2. 8%) and12(1. 4%) respectively. Among the T-cell lymphomas, mycosis fungoides, anaplastic large-cell lymphomas of T/null-cell type, and peripheral T-cell lymphomas accounted for 6%,4. 3%, and 2. 9% of all cases, respectively. **Conclusions.**To our knowledge, this is the biggest NHL series to be reported inJordanto date. Non-Hodgkin lymphoma appears to constitute a smaller share of the lymphoma burden inJordan, as opposedEuropeand theUS. Clinico-pathological features, however, show important differences from those described in the rest of the world. Follicular lymphoma and mantle-cell lymphoma are less common inJordancompared toEuropeand theUSA. Peripheral T-cell lymphomas and T/NK-cell lymphomas of nodal and extranodal nasal types, which are common in many other Asiancountries, are also less prevalent. . DLBCL, as a result, formed a bigger proportion of NHL inJordan.

1691

WITHDRAWN

1692

HEPCIDIN LEVELS IN DIFFUSE LARGE B CELL NON-HODGKIN LYMPHOMA

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Background. Anemia is a frequent sign in patients with diffuse large B cell lymphoma (DLBCL) at diagnosis, and it is associated with unfavourable patient characteristics. A potential cause for anemia in Non-Hodgkin's lymphoma (NHL) is the abnormal iron-utilization, characteristic for the anemia of chronic disease, mediated from the liver-produced acute-phase peptide hepcidin. Overproduction of hepcidin leads in fact to iron-limited erythropoiesis, and inflammatory cytokines increase the expression of hepcidin. We recently showed that in patients with Hodgkin lymphoma IL-6 up-regulates hepcidin and elevated hepcidin levels resulted in iron-restriction and signs of anemia of chronic inflammation (Hohaus et al, J Clin Oncol, 2010; 28:2538). **Aims.** To assess the contribution of some principal players for erythropoiesis to the development of anemia in DLBCL: the iron-store regulator hepcidin, the hormone erythropoietin, and the inflammatory cytokine IL-6. Associations to other patients characteristics were also analyzed. **Methods.** We studied 53 patients with DLBCL (median age 61 years, range 16-78 years; 28 females and 25 males); a group of 24 healthy individuals (median age 41 years, range 18-63 years; 13 female, 11 males) was used as control. Plasma samples were analyzed for hepcidin levels using a combination of weak cation exchange chromatography and time-of-flight mass spectrometry (TOF MS); cytokine levels were determined using ELISAs and parameters of iron metabolism and acute phase reaction were all determined in the central laboratory of the Catholic University. Associations with patient characteristics were analyzed using standard statistics (STATA 10). **Results.** At diagnosis, 29 of 53 (55%) DLBCL patients had haemoglobin (Hb) levels <12 g/dl. Hepcidin plasma levels were significantly higher in DLBCL patients when compared to controls, independent of the presence of anemia (p=0. 001). Higher hepcidin levels were observed in patients with more aggressive disease characteristics, as elevated LDH levels (p=0. 0004), stage IV disease (p=0. 01), presence of B-symptoms (p=0. 03), and IPI score > 2 (p=0. 005). Hepcidin levels strongly correlated to ferritin (β=0. 77, p<0. 001), and to IL-6 levels (β=0. 3 p=0. 03), and inversely correlated to iron-binding capacity (β=-0. 36 p=0. 04), but not to haemoglobin and erythropoietin values. Haemoglobin values inversely correlated to IL-6 (β=-0. 36, p=0. 009) and erythropoietin levels (β=-0. 36, p=0. 009), however the observed/predicted erythropoietin levels were lower than 0. 8 in 26/29 (90%) of anemic DLBCL patients compared to 10/24 (42%) of non-anemic patients (p=0. 01) indicating a blunted erythropoietin response in anemic patients. In a logistic regression analysis including hepcidin, IL-6, and the O/P ratio of erythropoietin, Il-6 levels retained their significant impact on the risk of anemia (OR=1. 68, 95% C. I., 1. 01-2. 79; p=0. 044). **Conclusions.** Our findings suggest that hepcidin levels are associated with parameters of disease activity in DLBCL and are potentially up-regulated by IL-6. Elevated hepcidin levels result in iron-restriction, however a blunted erythropoietin response and, in particular, elevated Il-6 levels are associated with anemia in DLBCL.

1693

CONCORDANT BONE MARROW INVOLVEMENT MAINTAINS AN ADVERSE PROGNOSTIC SIGNIFICANCE IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH R-CHOP

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Background. The improvement in treatment outcomes of diffuse large B-cell lymphoma (DLBCL) observed following the introduction of Rituximab has altered previous views about risk assessment. Approximately 10% to 25% of patients (pts) with DLBCL exhibit bone marrow involvement with lymphoma at the time of diagnosis. Concordant bone marrow involvement has generally been associated with a poorer outcome but the impact of discordant involve-

ment remains less clear. **Aims.** The aim of this study was to examine the prognostic impact of (BM) involvement on response rate (RR), overall survival (OS) and progression-free survival (PFS) in 178 patients with de novo DLBCL. **Patients and Methods.** Between 2000 and 2010 we retrospectively analyzed 178 untreated DLBCL pts, newly diagnosed at our centre, with or without (BM) involvement. The median age was 59 yrs (16-89 yrs), 109 (61%) were males, 75 (43%) had B symptoms, 27 (15%) had extranodal dissemination >1, 49 (28%) had a high IPI score. The treatment protocol for front-line therapy included rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). Progression-free (PFS) and overall survival (OS) were calculated according to the absence or presence of (BM) involvement and the presence of histological discordance or concordance between the primary site and the bone marrow. **Results.** A total of 41 (23%) of 178 pts had bone marrow involvement, concordant in 16 (9%) and discordant in 25 (14%). Median follow-up was 30 months (range, 1 to 120). The 41 pts with bone marrow involvement had a poorer prognosis compared with the group without (3 year progression-free survival, 56% vs 81% - p<0. 05).) PFS was inferior in those with concordant (p<0. 001) and discordant (p<0. 05) involvement, while OS was inferior only in those with concordant involvement (p<0. 001). Concordant involvement was associated with elevated lactate dehydrogenase, extranodal dissemination>1, IPI>2, performance status >2 and elevated beta 2 microglobulins. Complete remission (CR) rates were inferior in concordant bone marrow involvement as compared to discordant or no involvement (48% vs 59% vs 64%). **Conclusions.** Our data show that concordant, but not discordant bone marrow involvement, could represent a possible predictive factor of poor prognosis, which would help to identify a high risk subgroup of newly diagnosed DLBCL. To confirm these results, further large-scale and prospective studies will be required.

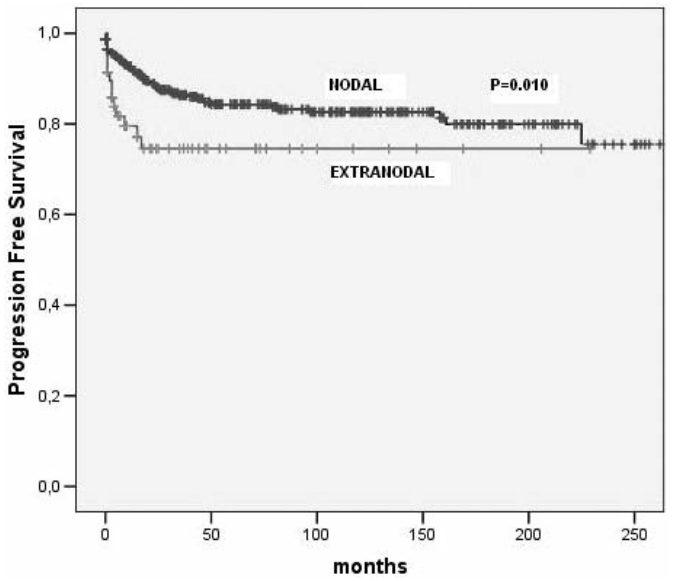

Figure 1. Progression free survival in patients with Hodgkin's lymphoma: nodal *vs* extranodal disease.

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THE GMALL-B-ALL/NHL PROTOCOL IS AN EFFECTIVE SALVAGE TREATMENT IN REFRACTORY AND RELAPSED AGGRESSIVE B-CELL LYMPHOMA

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Background. The prognosis of refractory and relapsed aggressive non-Hodgkin lymphomas (NHL) is poor. After first-line treatment, platinum based salvage therapy followed by high-dose chemotherapy and autologous stem-cell transplantation may rescue 20% to 45% of patients. The B-ALL/NHL protocol, originally designed for the treatment of Burkitt lymphoma by the German Acute Lymphoblastic Leukemia study group, may be an alternative salvage regimen. Its main constituents are hyperfractionated alkylating agents (cyclophosphamide, ifosfamide) and high doses of protractedly infused antimetabolites (methotrexate, cytarabine) able to cross the blood - brain barrier. **Aims.** This study analysed the efficacy and toxicity of the B-ALL/NHL protocol in refractory and relapsed patients with aggressive NHL. **Methods.** This is a single center, retrospective analysis of outcomes in relapsed and refractory patients treated with the B-ALL/NHL protocol (with or without rituximab) between 1994 and 2010. 26 diffuse large B-cell lymphomas (DLBCL incl. variants and subtypes; median age 58 years (27-78), 7 transformed B-NHL (tB-NHL; median age 67 years (36-71), 3 T-cell-lymphomas (TCL; median age 58 years (29-63) and 6 mantle-cell lymphomas (MCL; median age 60 years (38-65) were analysed for overall survival (OS) and toxicities. **Results.** The main factor affecting response was the number of previous treatment lines. Three-year OS in DLBCL patients after a maximum of one previous treatment line was 44. 4%, compared with 7. 7% in patients in later treatment phases (p<0. 05). OS in patients refractory to first line treatment (progressive under first line therapy or remission lasting less than 6 months) was 20%. Three-year OS in tB-NHL and in MCL patients was 28% and 0%, respectively. In this cohort, 21% of treatment cycles had to be delayed, mainly due to prolonged bone marrow toxicity, neutropenic fever and mucositis. Grade 3 and 4 toxicities related to infectious causes were observed in 85. 5 % of patients. Treatment-related mortality was 4%. **Summary and Conclusions.** The B-ALL/NHL protocol is an effective salvage regimen for DLBCL and tB-NHL refractory to front line therapy or in first relapse. Treatment-related morbidity, mainly due to infectious complications, is high, but manageable and reversible. Treatment related mortality is not in excess of other salvage regimens.

1695

LYMPHOPENIA PREDICT FOR THE RISK OF FEBRILE NEUTROPENIA AFTER CHEMOTHERAPY

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Background. Febrile Neutropenia (FN) is the most serious hematological toxicity of cytotoxic chemotherapy. Individual parameters of the patient contribute significantly to the risk of FN in addition to the type and dose of chemotherapy. **Methods and Aims.** We analyzed data of 193 cycles of chemotherapy given for 42 patients with aggressive lymphoma to evaluate the impact of lymphopenia on day 1, day 5, and day 8 on the occurrence of FN. **Results.** The median age was 33 years (15-68) with male/female sex ratio of 1. 8. Half of patients had classical Hodgkin lymphoma, one third with diffuse large B cell lymphoma and 12 % with T cell lymphoma. Stage I, II, III, and IV were observed in 2. 4%; 40. 5%; 21. 4%; and 35. 7% respectively. Cycles were composed of: 74 courses of BEACOPP, 116 cycles of CHOP and CHOP like and 3 of IGEV. FN occurred more frequently in female than in male (34. 6% vs 19 %; p= 0. 015). Stage IV was significantly associated with FN than other stage (36% vs 21. 6%; p=0. 045). FN was observed in 25. 4% of cases. We study the correlation between FN and lymphopenia ≤ 700/mm^3 on day 1, day 5, and on day 8. We noted an increase of the risk of FN by 2 fold (OR 2. 2) when lymphocytes count ≤700/mm^3 on day 1. Data for day5 were available only for 57 courses, explained the absence of impact of this parameter. Lymphopenia ≤ 700/mm^3 on day 8 was associated with a higher risk of FN (OR=2. 4; p=0. 02). Logistic regression study found that lymphopenia on day 8(≤ 700/mm^3) was not an independent factor to predictive FN. Moreover analyzes showed that this factor was significantly associated with increase of hematotoxicity (p=0. 02), duration of aplasia and of antibiotic therapy. We also concluded that hematotoxicity observed in the first cycle was a predictor factor for toxicity in later cycles (p=0. 03). **Conclusions.** Lymphopenia ≤ 700/mm^3 on day 1 and /or on day 8 of chemotherapy allowed selecting a group of patient candidate for prophylactic measures to FN.

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SMOKING AND NON-HODGKIN'S LYMPHOMA

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Background. Non-Hodgkin lymphoma (NHL) is the fifth most frequently diagnosed cancer. The incidence of non-Hodgkin lymphoma has risen by at least 100 percent over the past five decades especially in the western, but the reason is not clear. Tobacco smoking is the important cause of several malignancies. Different studies show various effect of smoking on Non-Hodgkin lymphoma. To evaluate this effect, a case control study was carried out in NHL patients in a cancer center in Mashhad, Iran. **Methods.** We conducted a case-control study on NHL in Mashhad, which includes 170 NHL cases (mean age 52 years) were admitted to reference hospitals and outpatient clinic, and 190

controls (mean ages 46 years) were admitted for a wide spectrum of nonmalignant, non tobacco-related conditions. Trained interviewers administered a structured questionnaire to cases and controls during their visit from 2008 to 2010. **Results.** We found that if we only compare smoking with non-smoking, there is no significant effect of smoking on Non-Hodgkin's Lymphoma. In control group 21. 8%, and in case group 24. 7% were smoker (p=0. 521). But if we compare the amount of smoking, there was significant difference between case and control groups. 45% of patients smoke more than 15 cigars per day, but only 7. 9% of control group were smoked more than 15 cigar per day (p=0. 000). In this study the subtype of NHL based on WHO classification, in 38% of patients was diffuse large cell lymphoma and only 1. 4% was follicular lymphoma. **Conclusions.** Based on our study, only smoking is not a risk factor for Non-Hodgkin Lymphoma, but heavy smokers (smoke more than 15 cigars per day) are at risk for Non-Hodgkin's Lymphoma.

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R-ICE AS SALVAGE THERAPY FOR RELAPSED DIFFUSE LARGE B-CELL LYMPHOMA WITH PRIOR EXPOSURE TO RITUXIMAB: A MULTI-CENTER, PROSPECTIVE PHASE II TRIAL

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Background. The prognosis of patients with relapsed diffuse large B-cell lymphoma (DLBCL) after rituximab-containing first-line therapy is relatively poor. Rituximab, ifosfamide, etoposide, and carboplatin (R-ICE) was proved to be an effective salvage regimen for relapsed DLBCL. **Aims.** We aimed to prospectively evaluate the efficacy and safety of R-ICE in patients with prior rituximab exposure in a multi-center trial setting. **Methods.** DLBCL patients who had disease relapse after 6-8 cycles of first-line therapy of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) could be enrolled in this trial. Patients without complete response (CR) or complete response unconfirmed (CRu) after R-CHOP were ineligible. Three cycles of R-ICE (rituximab 375 mg/m^2 day 1, ifosfamide 1600 mg/m^2 days 2-4, carboplatin area under the curve = 5 [maximum dose, 800 mg] day 3, and etoposide 100 mg/m^2 days 2-4) were given to eligible patients. For responding and young (less than 60 years) patients, high-dose therapy (HDT) and autologous stem-cell transplantation (ASCT) were recommended, but not mandatory in the protocol. For remaining patients, selections of subsequent treatment were at the discretion of their attending physicians. The primary endpoint of this trial was overall response rate and secondary endpoints included progression-free survival (PFS), overall survival (OS) and safety. **Results.** Thirty-two patients were enrolled with a median age of 55 years (range, 27 to 68). Other major patient characteristics were shown in Table 1. After 3 cycles of R-ICE, 25 (78. 1%) patients responded to the therapy. The CR/CRu and partial response (PR) rate were 50% and 28. 1%, respectively. Three patients developed early disease progression after 1 or 2 cycles. One patient refused further treatment after 1 cycle and was lost of follow-up. Among responding patients, 5 patients underwent HDT followed by ASCT and 9 patients received another 1 or 2 cycles of R-ICE. Other subsequent therapy included rituximab maintenance (1 patient), other salvage chemotherapy (5) and radiotherapy (4). Bone marrow suppression was the major toxicity. The rates of grade 3/4 leucopenia, anemia and thrombocytopenia were 37. 5%, 15. 6% and 37. 5%, respectively. Six (18. 8%) patients developed febrile neutropenia, but none died consequently. With a median follow-up time of 12 months (range, 7. 7 - 16. 3), 1-year PFS and OS rate were 53. 4% and 70. 7%, respectively. Sub-group analysis demonstrated that international prognostic index (IPI) before enrollment correlated with survival. In patients with IPI 0-2, 1-year PFS and OS rate were 67. 4% and 77. 9%, which were higher than those in patients with IPI 3-5 (18. 2% and 51. 4%) with statistically significant *P* values (0. 04 and 0. 033). Through multivariate analysis, IPI was also found to be the sole independent prognostic factor regarding PFS and OS. For patients with a longer relapse time (more than 12 months) after first-line therapy, there was a trend of significantly improved 1-year PFS and OS rate (71. 8% and 82. 1%) as compared with early relapse patients (35. 1% and 58. 3%). **Conclusions.** In relapsed DLBCL patients with prior rituximab exposure, R-ICE is still an effective salvage regimen with manageable toxicities. IPI remains a clinically significant prognostic factor in second-line setting.

Table1. Patients characteristics.

	No.	%
Total number	32	100
Median age, years (range)	55 (27-68)	
Gender		
Male	20	62.5
Female	12	37.5
ECOG performance status		
0-1	30	93.8
2	2	6.2
Ann Arbor stage		
I-II	8	25
III-IV	24	75
B symptoms		
Yes	6	18.8
No	26	81.2
Lactate dehydrogenase		
Elevated	10	31.3
Normal	22	68.7
International Prognostic Index (IPI)		
0-1	15	46.9
2	9	28.1
3	6	18.8
4-5	2	6.3
Relapse time after first-line therapy, months		
< 12	19	59.4
⩾12	13	40.6

1698

NEW PROGNOSTIC MODEL IN FOLLICULAR NON-HODGKIN LYMPHOMA TREATED WITH RITUXIMAB + CHOP: ASSOCIATION OF COMORBIDITIES WITH OVERALL SURVIVAL

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Background. The incidence of follicular non-Hodgkin lymphoma (NHL) increases with age and a high prevalence of comorbid conditions has been reported in these patients. So far, risk assessment in NHL has been mainly based on disease status. **Aims.** The objective of this study was to determine the prognostic impact of comorbidity on the survival of patients with NHL with the aim of developing novel tool for risk assessment. **Methods.** We conducted a retrospective cohort study of 65 patients with follicular NHL who received Rituximab+CHOP, identified in Registry of the Serbian Lymphoma Group. The comorbidity scores as Adult Comorbidity Evaluation-27 (ACE-27), Cumulative Illness Rating Scale (CIRS) and hematopoietic cell transplantation comorbidity index (HCT-CI) was used to assess concurrent presence of nonmalignant diseases. Data on demographics, disease status, treatment, and outcome were extracted from detailed review of the patients' medical charts and laboratory values at diagnosis and during the course of the disease. Kaplan-Meier methods and univariable and multivariable Cox regression were used to assess survival. A prognostic model incorporating baseline comorbidities was developed to predict survival. **Results.** According to ACE-27 and HCT-CI scales, comorbidity was present in 62% and 40% of patients in the followed cohort, respectively. Overall mean survival was 37,4 months. In univariate Cox regression analysis, both comorbidity indexes - ACE-27 and HCT-CI had a significant impact on overall survival (p=0,039; RR=2,197; 95%CI for RR 1,040- 4,641 and

p=0,039; RR=2,197; 95%CI for RR 1,040- 4,641) along with with FLIPI prognostic index (p=0,039; RR=2,197; 95%CI for RR 1,040- 4,641). CIRS scale were not found to affect the risk of overall death. Median overall survival time between ACE groups (none/mild *vs.* moderate/severe) was 39 *vs.* 30 months, respectively (p=0,056) while in HCT-CI groups (HCT-CI < 2 vs ≥ 2) was 37 vs 31 months (p=0,063). On multivariate regression analysis, only the HCT-CI index remained a prognostic factor (p=0,027; RR=1,666; 95%CI for RR 1,061-2,616) independent of FLIPI. **Conclusions.** Comorbidities have a significant impact on the outcome of patients with follicular NHL treated with Rituximab. A comprehensive assessment of the severity of comorbidities through the HCT-CI and ACE-27 scales helps predict survival in patients with follicular NHL.

1699

ACUTE RENAL FAILURE IN BURKITT'S LYMPHOMA PATIENTS UNDERGOING BL-M-04 TREATMENT PROTOCOL

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Background. Burkitt's Lymphoma (BL) - B -cell lymphoid neoplasm with high proliferative index approximates 100%, chromosomal abnormalities (t(8;14)(q24;q32), rarely - t(2;8)(p12;q24), t(8;22)(q24;q11)). Renal failure in BL patients can be caused by the tumor itself and appears during chemotherapy. The National Research Center for Hematology uses original high intensive protocol BL-M-04. **Aims.** To analyze reasons of Acute Renal Failure (ARF) and treatment results in BL patients. **Methods.** 13 previously untreated patients were included in our study (7 males and 6 females, median age of 24, spanning 15 to 57 years old). All patients had c-myc rearrangements and ARF. Patients with c-myc rearrangements, but without ARF comprised the control group (36 patients). All patients underwent chemotherapy in the National Research Center for Hematology between May 2003 and August 2011. Treatment was based on modified NHL-BFM-90 protocol - BL-M-04, including high dose of methotrexate (MTX) (1,5 g/m²), cytarabine (2 g/m²). The stage of disease was established according to Murphy S. B. classification. III, IV stage was diagnosed in 5 and 1 patients respectively. B-acute lymphoblastic leukemia (L3) was diagnosed in 7 patients. The ratio between patients with bone marrow involvement and those without was7:13 in the main group,9:36 in the control group. The differences between the compared groups were tested by non-parametric statistical analyses (Mann-Whitney U-test). The data was analyzed using SPSS, ver. 16. 0. Differences were considered significant at p ≤0. 05. **Results.** 12 patients from 13 were diagnosed with ARF at the moment of admission to hospital. In 5 patients it became greater after treatment started. In 1 case AFR appeared for the first time after chemotherapy began. The reasons for ARF at the time of admission to hospital were tumor lysis syndrome (TLS) and/or tumor kidney infiltration (5, 6 events respectively). 2 patients had urinary obstruction. After treatment started, TLS and/or MTX toxicity were the main causes (4 and 3 events respectively). Renal function impairment was observed when MTX was administered before ARF resolved (3 events), independently of dosage. Later MTX was administered only after AFR had disappeared. ARF resolved in a short time after treatment was started: during the pre-phase or first course A of chemotherapy (5 and 8 events respectively). Chemotherapy in BL patients with AFR was associated with an earlier myelotoxic agranulocytosis, (beginning on the 3rd day after chemotherapy finished, compared with the 5th day in the control group) (p=0. 04), longer myelotoxic agranulocytosis duration (12 days, compared with the control group - 7 days) (p=0. 04), higher mucositis degree (p=0. 05). Although 5 patients died (4 due to infectious complications, 1 due to thrombocytopenia), clinical remission was achieved in all patients. **Conclusions.** Hematological and gastrointestinal toxicity degree in BL-M-04 protocol is significantly higher in BL patients with ARF in comparison with the BL patients without ARF (p=0. 04). MTX should be administered only after ARF has resolved. Mortality in BL patients with ARF is significantly higher than in BL patients without ARF (33 % vs 10%, p = 0. 05).

1700

ASSESSMENT OF BONE MARROW INVOLVEMENT BY FDG-PET/CT IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Background and Aims. Bone marrow biopsy (BMB) is an essential part of the staging of follicular lymphoma (FL) patients as 40-60% of patients have involvement of bone marrow (BM) at presentation. However, given the patchy infiltration of BM in patients with FL, BMB -an unpleasant investigation- can miss disease not present in the biopsy area. Several studies evaluating the role of FDG-PET in assessing BM involvement have relied on visual analysis alone, which has proved inadequate. Against this background, the aim of this study was to assess the additional value of semi-quantitative assessment in predicting BM involvement in FL. **Patients and Methods.** A single centre retrospective analysis of 41 patients with grade 1-3a FL (median age: 64 years, range: 30 - 87; previously untreated: 30, at relapse: 11), who underwent FDG -PET/CT and unilateral BMB as part of the staging assessment is reported. The last treatment for patients included at relapse was given at least 3 months (range: 3 - 54) prior to the staging investigations. BM uptake was assessed both visually and with semi-quantitative analysis. On visual analysis, BM uptake was compared to the mediastinal blood pool (MBP) as a reference. Semi-quantitative analysis involved measuring the maximum SUV at the sternum, both iliac blades and the T12 vertebra. The single highest SUVmax, the average SUVmax (SUVav) for the 4 sites and the ratio of SUVav/MBP were compared to the BMB result by Welch test on SPSS. Optimal SUVmax, SUVav and SUVav/MBP cutoffs were defined by Receiver operating characteristic (ROC) curves. **Results.** Sixteen patients had a positive BMB. On visual analysis of the FDG-PET/CT, 7 patients had BM involvement (focal lesions in 6). Two patients deemed to have possible bone involvement on visual analysis of the FDG-PET/CT had negative BMB. Visual analysis had a sensitivity and a specificity of 31% and 92%, respectively, whereas the sensitivity and the specificity for SUVmax ≥2. 5 was 56% and 84%; those of SUVav≥2 were 63% and 96%, and the sensitivity and specificity of SUVav/MBP ≥1 were 88% and 80%, respectively. **Conclusions.** Visual assessment of FDG-PET/CT has a high specificity but a low sensitivity to detect BM involvement in FL patients. In contrast, semi-quantitative assessment can improve the sensitivity of FDG-PET/CT to predict BM involvement from 31% to 88%. Were these results confirmed in further prospective studies, they may help to select patients who might benefit from having a BMB because of likely involvement, with the potential to consider avoiding BMB in the rest.

Table 1.

Methods for assessment of BM involvement		BMB +VE	BMB -VE
FDG-PET/CT +VE on visual analysis (patients)		5	2
FDG-PET/CT – VE on visual analysis (patients)		11	23
SUV max (mean)	p= 0.02	2.8	2.0
SUV av (mean)	p=0.004	2.3	1.6
SUV av/MBP (mean)	p=0.001	1.4	0.9

1701

THE IMPACT OF COMORBIDITY BURDEN ON THE OVERALL SURVIVAL IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background. After the introduction of R-CHOP as standard treatment of Diffuse Large B-cell Lymphoma (DLBCL), treatment results significantly improved. Still, there are patients who didn't have benefit from this. The main task of investigators focused on prognostic factors is to identify the patients at high risk for poor outcome. Modern oncological studies includes a comprehensive evaluation of comorbidity as an important predictor of the outcome because of the role of competing risk. **Aims.** The objective of this study was to assess the prognostic impact of the Adult Comorbidity Evaluation-27 (ACE), Modified Cumulative Illness Rating Scale (M-CIRS) and Hematopoietic Cell Transplantation -specific comorbidity index (HCT-CI) on the overall survival in patients with DLB-

CL. **Methods.** This retrospective study included 145 patients diagnosed with DLBCL in the period May 2008-August 2010 in three centers, identified in DLBCL Register of the Serbian Lymphoma Group. All the patients were treated with R-CHOP in the front line therapy. Information on comorbidity was extracted from patient's history and ACE-27, M-CIRS and HCT-CI comorbidity indexes were calculated. Univariate and multivariate survival analyses were performed using Cox's proportional hazards regression models. **Results.** The median age was 56. 3±14. 1. Overall mean survival was 35. 4±1. 5 months. According to both ACE-27 and HCT-CI categories, patients with severe combidities had significantly lower overall survival, respectively (p=0. 007 and p=0. 030). The overall survival of patients with M-CIRS over the median for the selected group didn't differ from patients with M-CIRS below the median. Furthermore, in univariate regression analysis International Prognostic Index (IPI), Revised-IPI (R-IPI) and age adjusted IPI (aaIPI) were identified as predictors of overall survival, respectively (p<0. 001; p<0. 001; p<0. 001). Final multivariate prognostic model included aaIPI and HCT-CI as the most important predictors of overall survival (P < 0. 001 and p=0,012). **Summary and Conclusions.** A comprehensive assessment of the severity of comorbidities through the ACE-27 and HCT-CI comorbidity indexes helps predict overall survival, while the aaIPI and HCT-CI are the most important predictors of overall survival in patients with DLBCL.

1702

OUTCOME OF PATIENTS WITH PCNSL TREATED OUTSIDE CLINICAL TRIALS: A RETROSPECTIVE STUDY

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Background. Primary CNS lymphoma (PCNSL) accounts for approximately 4% of newly diagnosed central nervous system tumours. The current knowledge and guidelines are mainly based on results obtained in clinical trials. However, due to the often poor performance status and cognitive impairment, many patients are treated outside studies. **Aims.** The aim of this study was to get more insight into the nature, course of disease and outcome of HIV negative patients with PCNSL who are treated outside clinical trials. **Methods.** This is a retrospective study of all HIV negative patients with PCNSL diagnosed between 2000 and 2010, treated outside clinical studies in the Antoni van Leeuwenhoek hospital and Academic Medical Center in Amsterdam. The data were retrieved from the registries of both hospitals. The following data were investigated: age, sex, performance status (PS), comorbidities, presence of other immunodeficiencies than HIV infection, presenting symptoms, the PCNSL prognostic score at presentation, pathology, treatment, response, duration of response, acute and late toxicities, overall, event- and recurrence free survival (OS, EFS, RFS) and cause of death. RFS was considered only for patients with response to chemo- and/or radiotherapy. RFS was defined as time from diagnosis to date of recurrence or death, whatever happened first; OS as time from diagnosis to death and EFS as time from diagnosis to any disease failure for all patients. The study was approved by the Ethics Committees of both hospitals. **Results.** Fifty-two patients were identified. The characteristics and treatment are summarized in the Table 1.

Table 1.

Characteristics	No. of patients
Total	52 (27 male)
Median Age (years)	64,5 (range 43-83)
Performance status: 0-2 respectively (resp.) 3-4	33 resp. 19
PCNSL Prognostic score	
Complete	25
0-2 resp. ≥ 3	10 resp. 15
Incomplete (no cerebrospinal fluid data: 25; LDH unknown :2)	27
0-2 resp. ≥ 3	17 resp. 10
Presenting symptoms	
Focal neurological deficits	40
Cognitive dysfunction	26
Decreased consciousness	19
Epilepsy	17
Headache	15
Behavioural changes	14
Walking disturbances	12
Nausea/vomitus	11
Number of sites involved	
Single resp. multiple	29 resp. 23
Location of sites	
Supratentorial tumor	43
Infratentorial tumor	2
Both supra-and infratentorial tumor	6
Leptomeningeal involvement	6
Ocular involvement	1
Time between first symptoms and diagnosis in months	1,5 (range 0-34)
Time between diagnosis and treatment in months	0,5 (range 0-20)
Treatment and response	
High dose MTX based chemotherapy alone	16: 6 CR; 2PR; 4 PD; 4 NE (NE=not evaluable, died before evaluation)
Radiotherapy (RT) alone	10: 4 CR; 4 PR; 1 PD; 1 NE (1 lost for FU before evaluation)
High dose MTX based chemotherapy and RT	18: 12 CR; 3 PR; 2 PD; 1 SD
No treatment	8

In 14 patients, no histological confirmation of the diagnosis PCNSL could be retrieved. The reasons were: poor PS, danger of severe neurological damage due to biopsy, high intracranial pressure with danger of cerebral herniation. All patients with histological verification had a diffuse large B-cell lymphoma. In 27 patients, no complete PCNSL prognostic score could be calculated mainly due to the risks to do a lumbal puncture. All patients were treated with corticosteroids: 32 patients had a clinical response. 8 patients received no further chemo- or radiotherapy due to poor PS and rapid deterioration despite corticosteroids. With a median follow up of 63. 1 months, 34 patients had died: 29 due to PCNSL (14 from primary tumor, 15 after recurrence), 4 due to toxicity of chemotherapy and 1 due to epileptic seizure while in CR. Four patients were lost for follow-up (FU). The date of last contact was chosen to determine the OS and RFS in patients lost to FU. With a median FU of 63. 1 months, median RFS for 32 responding patients was 23. 8 months (95% CI 15. 9-not applicable), median OS for all patients 24. 4 months (95% CI 11. 5-39. 8), median EFS 14 months (95% CI:7. 3-24. 4). **Summary and Conclusions**. The results are in line with those published in prospective series. The inability to obtain liquor in 25/52 patients indicates that protein content of liquor is a less usable prognostic factor. Lack of histology in 14 patients excluded them from trial participation. In order to obtain more knowledge about PCNSL, a central international registry could be of value.

1703

DIFFUSE LARGE B-CELL LYMPHOMA IN KOREA: ITS DISTINCT EPIDEMIOLOGY AND CLINICAL FEATURES

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Background. Non-Hodgkin lymphoma (NHL) is the tenth common malignancy and WHO age-standardized incidence rate is 8. 3 per 100,000 for both sex in Korea. Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL with heterogeneous clinical presentations and biological behaviors but there are rare collective data on its epidemiology and clinical features. **Aims.** This study aims to review comprehensive clinical features and to determine factors affecting survival of DLBCL in Korean.

Table 1. Extranodal involvements at initial diagnosis.

Extranodal sites (total 563 patients, total 889 sites)	Frequency	%, total sites [a]	%, total patients [b]
Gastrointestinal	*221*	*24.9*	*39.3*
Stomach	114	12.8	20.2
Small bowel / Ileocolic or ileocecal	45/9	5.1/1.0	8.0/1.6
Colon/Rectum	18/6	2.0/0.7	3.2/1.1
Multiple GI	29	3.3	5.2
Head and neck	*184*	*20.7*	*32.7*
Waldeyer's ring	140	15.7	24.9
Other head and neck	44	4.9	7.8
Soft tissue	*128*	*14.4*	*22.7*
Skin	24	2.7	4.3
Bone	67	7.5	11.9
Muscle and others	37	4.2	6.6
Bone marrow	*77*	*8.7*	*13.7*
Lung and pleura	*86*	*9.7*	*15.3*
Lung	44	4.9	7.8
Pleura	42	4.7	7.5
Genitourinary organs	*55*	*6.2*	*9.8*
Kidney	25	2.8	4.4
Ureter/Bladder	1/2	0.1/0.2	0.2/0.4
Testis/Prostate	9/1	1.0/0.1	1.6/0.2
Ovary/Adnexa/Cervix/Vulva	9/1/6/1	1.0/0.1/0.7/0.1	1.6/0.2/1.1/0.2
Liver	*40*	*4.5*	*7.1*
Breast	*13*	*1.5*	*2.3*
CNS (Brain, CSF, pituitary)	*13*	*1.5*	*2.3*
Brain	3	0.3	0.5
Orbit	5	0.6	0.9
CSF	1	0.1	0.2
Spinal canal	3	0.3	0.5
Pituitary	1	0.1	0.2
Other abdominal structure (Adrenal, omentum, pancreas, GB, biliary, peritoneum)	*70*	*7.9*	*12.4*

Methods. A total of 716 pathology-confirmed DLBCL patients in single center, between 2000 and 2009, were enrolled. We retrospectively reviewed clinical and survival data to find factors affecting clinical outcome. **Results.** 389 men (54. 3%) and 327 women were diagnosed DLBCL at a median age of 56 years (range, 15 - 92). 286 patients (39. 9%) were over 60 years old and 36 (5. 1%) were less than 30 at diagnosis. 57 patients (8. 0%) were ECOG performance status more than 1, 404 (56. 4%) were Ann Arbor stage of III or IV, 191 had extranodal involvement more than 1 (26. 7%) and 416 (65. 4%) had abnormal initial serum lactate-dehydrogenase (LDH). Also, 35 in 474 patients (7. 4%) had chromosomal abnormality in bone marrow. 584 patients received chemotherapy alone, 15 radiotherapy alone, 65 chemotherapy followed by radiotherapy. The other 23 and 29 received surgery and best supportive care, respectively. A total of 556 patients were identified to have 1631 lymph node (LN) involvements (Most common were abdominal followed by cervical and mediastinal LNs, but no cases of infraclavicular and popliteal LNs were found). And 563 patients had 889 extranodal sites (Most common were gastrointestinal organs followed by head and neck area. Rare presentations such as prostate, female adnexa, vulva, pituitary and cerebrospinal fluid were found). After median follow-up of 41. 9 months (range 0. 1 - 140. 9), 716 patients showed 5-year survival rate of 62%. 680 evaluable patients revealed response rate of 89. 9% (Rituximab added cases were 92. 2% and no rituximab cases 86. 5%). Adding rituximab to CHOP chemotherapy did not show statistically significant gain in overall survival and event free survival. Prognostic models such as International prognostic index (IPI), age-adjusted IPI, revised IPI were all well correlated with overall survival differences by risk stratification in Rituximab plus CHOP treated patients. In case of Revised IPI, 5-year survival rate in very good risk group (0 risk factor), good risk (1 or 2 risks), poor risk (more than 2 risks) respectively showed 84%, 65% and 50% (log rank p=0. 01). Multivariate analysis showed 4 factors were independent in our study: Age > 60 (HR = 2. 38, 95% CI 1. 71 - 3. 23, p < 0. 001), B symptoms (HR=1. 78, 95% CI 1. 27 - 2. 50, p = 0. 001), Abnormal LDH (HR 1. 66, 95% CI 1. 11 - 2. 48, p = 0. 013), Chromosomal abnormality in bone marrow (HR = 1. 72, 95% CI 1. 04 - 2. 86, p=0. 036). **Conclusions.** DLBCL is clinically heterogeneous, variable in treatment outcome. This single ethnicity study on epidemiology, clinical features and survival necessitates further clinical researches to refine our understandings of DLBCL.

1704

CAN LEVEL OF EBV-DNAEMIA PREDICT DEVELOP OF A POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER?

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Background. Post-transplant lymphoproliferative disorders (PTLDs) are a spectrum of lymphoid neoplasms, occurring in solid organ of immunosuppressed subjects or in bone marrow transplant recipients. PTLDs commonly derive from B-cell lineage and are associated with Epstein-Barr virus (EBV) infection. **Methods.** We retrospectively analyzed seven cases of PTLDs between 2000 and 2011. Five were males, one female, their mean age was 55 years (range: 15-72). Transplanted organs were heart (3 cases), kidney (3 cases), and liver (1 case). EBV DNA load was performed by quantitative polymerase chain reaction. **Results.** We labelled as early PTLD which occurred within 12 months after transplant. In our patients, median time after transplant to diagnosis of PTLD was 9 (early) and 190 (late) months. Histological and immunohistochemical analysis revealed diffuse large B-cell lymphoma in three subjects, multiple myeloma in a patient, anaplastic large cell lymphoma in one, marginal zone lymphoma in another one and lymphoplasmacytic lymphoma in the last patient. EBV serology was positive in five cases before transplant and in six after it. Median viral load was 2400 copies/mL (range: 400-10000 copies/mL). Currently, four patients are alive and three patients have died. *Discussion.* A review of the literature shows that more than 90% of EBV-related PTLDs commonly arise within one year after transplant. We observed a late PTLD onset in 4/6 cases. Two patients who developed PTLD within one year after transplantation had higher EBV-DNA load (median: 6000 copies/mL). Other four patients, with PTLD late onset, a lower viral load was detected (median: 550 copies/mL). It has been reported patients with undetectable or low EBV viral load within six months after transplant do not develop PTLDs, while a chronic high EBV load is a predictor of de novo or recurrent PTLDs. *Conclusions.* We think that PTLD late onset in our patients, could be related to their low EBV viral load. To date, it is not clear which threshold value of EBV-DNA can be considered predictive of PTLD development. High viral load or a rising trend could define higher risk patients, so frequent EBV load monitoring is mandatory.

1705

EXPRESSION OF KI 67AND CASPASE 3 WITH ANALYSIS OF SELECTED PROGNOSTIC FACTORS INFLUENCING ON OBTAINING 1ST AND 2ND DISEASE REMISSION IN PATIENTS SUFFERING FROM LYMPHOMAS

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Background. One of the most significant factor determining biology, clinical picture and effectiveness of therapy in lymphomas is the proliferation index, which can be characterized with Ki 67 expression. Main therapy in these diseases is chemotherapy, which efficacy could be in a large degree depending on both proliferation and apoptotic processes, as cytostatics are also functioning as drugs promoting apoptosis. Caspase 3 is of crucial importance in the process of apoptosis and though its expression may be correlated to response to chemotherapy. Prognostic factors for response to chemotherapy in these disorders shall help to individualize therapy and to improve its so far unsatisfactory efficiency. It particularly refers to recurrent disease, where it is often difficult to set a unified standard of care. **Aims.** Objective of this trial is to find more about expression of caspase 3 and it dependence to Ki 67 in lymphomas and to evaluate whether type of caspase 3 expression is a prognostic factor for response to treatment in particular types of lymphomas. Classical risk factors like IPI (International Prognostic Index), disease stage, age, serum LDH level and efficiency of first line treatment were also tested for influence on result of second treatments. In search for additional prognostic factors also other conventional variables like BMI (Body Mass Index), kind and quantity of extranodal involvement, degree of bone marrow involvement were tested for their significance. **Methods.** The project is retrospective, concerning patients with recurrent lymphomas treated in Lower Silesian Oncology Center and in Department of Haematology of Wrocław Medical University. Data was acquired by examination of expression of Ki 67 and caspase 3 with use of immunohistochemical analysis on tissue material from Department of Patology of Wrocław Medical University and Department of Pathology of Lower Silesian Oncology Center. Data was also acquired from patients' medical documentation. **Results.** Statistic analysis operates on group of 131 cases. In 68 patients it was possible to examine expression of Ki67and caspase 3. Low Ki 67 index (<5%) correlated with diffuse caspase 3 expression, while lymphomas with high proliferation index presented mild caspase 3 expression (p=0,04). Patients with Ki 67 (>30%) appear to have shorter OS counting for both 1st and 2nd line treatment (p=0,009) and tendency for worse PFS (p=0,09). Expression of caspase 3 did not influence ORR, PFS or OS in aggressive lymphomas, but it shows a trend of better OS in patient with mild expression in low grade lymphomas (see chart). Primary and restaged IPI, symptomatic disease, WHO PS status, LDH serum level, hemoglobin serum level, peripheral eosinophilia and number of CT cycles was found to influence PFS and/or OS, while extranodal involvement, BMI and restaged AnnArbor scale did not show significance. **Summary and Conclusions.** Expression of caspase 3 and probably also process of apoptosis correlates to proliferation index in lymphomas and might be a prognostic factor in indolent lymphomas. Prognostic factors for recurrent disease may differ from those known to be ones for first line therapy.

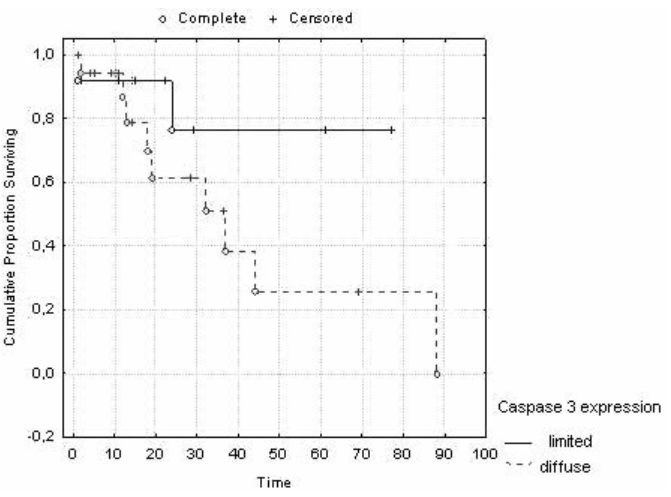

Figure 1. Cumulative proportion surviving (Kaplan-Meier).

1706

A RETROSPECTIVE REVIEW OF THE MANAGEMENT AND OUTCOMES OF ALL ELDERLY DLBCL PATIENTS DIAGNOSED OVER THE LAST FIVE YEARS AT THE NORTHERN CENTRE FOR CANCER CARE [NCCC]

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Background. 40% of patients diagnosed with DLBCL are over 70 years. Elderly patients have a poorer prognosis as they have a reduced response to standard chemotherapy but more significantly struggle to tolerate treatment. Standard treatment at the NCCC for DLBCL is 21 day CHOP-R for six cycles or reduced to 3 cycles with radiotherapy in limited stage disease. Extremely elderly patients i. e. > 80 years would be given a one meter squared CHOP-R dose. **Aims.** The aim of this case series is to assess treatment tolerability by examining chemotherapy dose intensity, adverse events, inpatient days, ITU admissions and deaths during treatment. **Methods.** All patients over 70 years who have been diagnosed with DLBCL in the past five years at the NCCC have been included in the retrospective analysis. Data was collected from a review of case notes and computerized records. **Results.** 53 patients were diagnosed with DLBCL in the past five years at NCCC. This group had an average age of 77. 6 years with a broad range of disease stages [stage I 20%, stage II 29%, stage III 22% and stage IV 29%] and IPI scores [low 18%, intermediate low 33%, intermediate high 29% and high 20%]. Overall response rates to treatment were CR 68%, PR 28% and PD 5%. At an average follow up of 31. 2 months the overall survival was 47%. Chemotherapy tolerability varied widely. 41% of patients received the chemotherapy treatment as planned however, 37% required either a dose reduction or omission due to adverse events and 22% of patients died during treatment. The inpatient burden also varied with 35% of patients not being admitted at all but of the 65% who required inpatient stay the duration of admission varied to a maximum of 156 days. Overall the average inpatient days required during chemotherapy was 20. 6 days with 6% of patients requiring ITU support. G-CSF prophylaxis became standard practice in 2009, however its introduction did not significantly reduce admission rates [p=0. 3]. Neutropenic sepsis was the commonest adverse event [38%] followed by peripheral neuropathy [20%] VTE [8%] and cardiac events [6%]. **Conclusions.** This retrospective review demonstrates that elderly patients with DLBCL are a heterogeneous group. It shows that elderly patients can be treated effectively and gain long term survival however, treatment toxicity is significant. Only 41% of patients could tolerate the planned chemotherapy and 65% of patients were admitted during treatment. Improvements in treatment tolerability through improved selection of patients for curative treatment, steroid pre-phases and individualization of therapy to patient co-morbidity will be essential with an aging population.

1707

HIGH FLIPI RISK FOLLICULAR LYMPHOMA: COMBINING HISTOLOGICAL GRADE, ERYTHROCYTE SEDIMENTATION RATE AND HYPOALBUMINEMIA CAN ADDITIONALY CONTRIBUTE IN PROGNOSIS

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Background. The widely accepted Follicular Lymphoma International Prognostic Index (FLIPI) divides patients in three risk group based on score of present unfavorable parameters (hemoglobin < 12 g/dL, age > 60 years, nodal sites > 4, elevated LDH, Ann Arbor Clinical stage 3 or 4). The estimated 5 years survival depending on FLIPI score is around 90% (0-1), 80% (2) and 50% (3-5), respectively. **Aims.** The aim of this study was to analyze the prognostic value of routinely used clinical and laboratory parameters which are not included in FLIPI index, in patients with high FLIPI risk. **Methods.** The retrospective analysis was performed on 57 patients with newly diagnosed follicular lymphoma (FL) with high risk according to FLIPI and with high tumour burden according to GELF94 criteria, diagnosed and treated at Clinic for Hematology, Clinical Center of Serbia, in the period April 2000-December 2006. In the first line, the patients were treated with R±CHOP or R±CVP. In the first relapse they were treated with fludarabine based regimens, R±FC or R±FND. The characteristics examined as possible risk factors were histological grade, bulky disease (the diameter of tumor >7 cm), ECOG performance status (ECOG PS), erythrocyte sedimentation rate (ESR) and serum albumin level. Receiver operating curve was used to determine the optimal cutoff value for ESR in prediction of overall survival for our group of patients. Survival functions were estimated using the Kaplan-Meier method and compared using the log-rank test. A multivariate analysis was performed to evaluate the potential predictive value of the examined characteristics as a risk factor. **Results.** The median follow up was 58 months (range 5-122 months). Bulky disease was present in 22 (38. 6%) patients. ECOG PS>1 on presentation had 18 (31. 6%) patients. Histological grade 1, 2 or 3a was present in 29 (50. 9%), 19 (33. 3%) and 9 (15. 8%) pts, respectively. Twenty-five patients (43. 9%) had ESR higher or equal than 45 mm/h. Serum albumin level below normal limit was observed in 28 (49. 1%). The patients with histological grade >1, ESR ≥45 mm/h and hypoalbuminemia had significantly lower overall survival (p=0. 015; p=0. 001; p=0. 008, respectively), while there was a trend toward worse overall survival in patients with ECOG > 1 (p=0. 075). Multivariate Cox regression analysis identified histological grade >1, ESR ≥45 mm/h and hypoalbuminemia as independent risk factors for poor outcome. Based on cumulative score of unfavorable prognostic factors, patients who had 0 or 1 unfavorable factors had significantly higher 5-year overall survival compared to patients with 2 or 3 risk factors (75% vs. 24. 1%, p=0. 000). **Summary and Conclusions.** Modern clinical researches are having the aim to individualize treatment approach based on risk for poor outcome. Our findings suggest that some newly diagnosed high FLIPI risk FL patients require more effective treatment, which should be defined in new prospective studies.

1708

RIT WITH 90Y-IBRITUMOMAB TIUXETAN IMPROVES THE RESPONSE IN AGGRESSIVE NON-HODGKIN LYMPHOMA

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Background. Add-on standard treatment of aggressive non-Hodgkin's lymphoma (NHL) with ^{90}Y Ibritumomab tiuxetan (^{90}Y-IT) (Zevalin®) has become an efficient alternative. **Aims.** The aim of this study is to present the analyzed updated results on treatment outcome obtained from our cohort treated with ^{90}Y-IT according to current clinical practice. **Subjects and Methods.** From September 2005 to February 2012, 19 aggressive lymphoma (AL) patients treated in the same center were included in a clinical protocol conducted by a multidisciplinary team. Inclusion criteria were: CD20+ LNH Mantle Cell Lymphoma (MCL) or Diffuse Large B cell Lymphoma (DLBCL) patients with neutrophils ≥ 1. 5 x 10^9/L, platelets ≥ 100 x 10^9/L, bone marrow lymphocytes CD20+ ≤ 25%. 7 patients were treated as consolidation of a first line chemotherapy; decision to treat was made by the multidisciplinary team according to characteristics of cases. All patients received 0. 4 mCi /kg IV of ^{90}Y-IT and response was evaluated 12 weeks after by PET/CT. Endpoints: objective response rate (ORR), progression free survival (PFS), overall survival (OS) and safety. Other clinical prognostic factors were observed to assess their possible influence upon treatment value.

Table 1. Means and medians for progression free survival time.

Type of Lymphoma	Mean[a]				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
MCL	27.213	4.482	18.427	35.998	27.000	7.379	12.538	41.462
DLBCL	39.000	6.062	27.118	50.882	.	.	.	.

a. Estimation is limited to the largest survival time if it is censored.

Table 2. Means and medians for overal survival time.

Type of Lymphoma	Mean[a]				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
MCL	58.500	.500	57.520	59.480	58.000	.	.	.
DLCBL	33.833	7.033	20.048	47.619	.	.	.	.

a. Estimation is limited to the largest survival time if it is censored.

Results. Until February 2012, 19 AL patients had received treatment with ^{90}Y-IT; 10 MCL (52. 6%), 9 DLBCL (47. 4%), 16 completed follow-up and were taken into analysis; M/F 10/9 (73. 6%/26. 4%); mean age 66. 9 (53-79) years for MCL and 53 (35-87) years for DLBCL; ECOG 0-1 82. 35%. According IPI score distribution: 0-1: 31. 25%, >1: 69. 75%. Previous therapy schedules: 1-2 (52. 6%), >2 (47. 4%). Median follow-up time: 46. 8 months. Mean estimated OS for MCL was 67. 6 months (50-84), median OS was 62 (37-86) and mean PFS was 27 months (95% CI: 18. 42-35. 99). For DLBCL mean OS was 85 months (53. 37-118. 45), median OS was 84 months (46. 44-121. 55), mean PFS was 39 months (95% CI: 27. 11-50. 88). Status before treatment was: relapsed but Complete Response (CR) after chemotherapy: 2 patients for MCL and 6 DLBCL;

relapse and refractory with active disease after chemotherapy (PR): 2 MCL and 1 DLBCL and as consolidation after first-line chemotherapy in CR: 4 MCL cases and 3 DLBC cases. 13 patients achieved CR, 8 MCL and 5 DLBCL; 2 MCL patients and 1 DLBCL only achieved PR/stabilization of the disease. Until now only 6 (40%) patients have relapsed, 4 MCL (27%). Mortality related to relapse was reported in only one MCL case that relapsed 15 months after treatment and died 3 years later. Safety: thrombocytopenia was the most frequent (42. 1%) hematological toxicity, median time to 2. 8 weeks, grade 3-4 only occurred in 21% of patients; neutropenia occurred in 31. 5%, and the median time for recovery was 3 and 2 weeks respectively. In 2 patients (10. 52%) red cell transfusion was required and 4 (21. 5%) needed platelet transfusion. The most frequent non hematological toxicity was asthenia. None secondary malignancies have been observed. **Conclusions.** In our experience ^{90}Y Ibritumomab tiuxetan (Zevalin®) is a safe and effective consolidation therapy in aggressive NHL, permitting achievement of sustained CR and prolonging PFS. Further studies on the impact of outcomes in this population are needed.

1709

LATE-ONSET NEUTROPENIA AFTER RITUXIMAB TREATMENT IN B-CELL LYMPHOMA: A SINGLE CENTRE EXPERIENCE

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Background. Recent studies have reported the occurrence of late onset neutropenia (LON) in 3%-27% of patients (pts) treated with Rituximab-based therapies. LON is defined as grade 3-4 neutropenia developing several weeks after Rituximab infusion; the aetiology is not completely understood. Several hypotheses have been proposed to explain this occurrence: infections, the production of antineutrophil antibodies, expansion of large granular lymphocyte (LGL) populations that may induce neutrophil apoptosis through Fas and Fas-ligand interactions. According to anther hypothesis, the interaction between B-cell recovery and stroma-derived factor-1 (SDF-1) inhibits neutrophil egress from the bone marrow. Immunoglobulin G Fc receptor polymorphism has also been suggested in the aetiology of LON. In addition, purine analogues, high dose therapy, AIDS are considered to increase the risk of LON. **Aims.** The aim of our study was to evaluate the incidence and clinical relevance of WHO grade 3/4 LON in pts treated with Rituximab-based therapy. **Methods.** We examined 238 B-cell lymphoma pts treated with Rituximab in our institution between 2008 and 2011; 124 (53%) of them had an indolent lymphoma. In9 pts(4%) the neutrophil count fell below 0. 5 x 10e9/L a few weeks after the last Rituximab infusion. Pts median age was 63 years (range 19-92). Histology was Follicular Lymphoma in 3 cases (33%), Marginal Zone lymphoma in 3 (33%), SLL in 2 (23%) and DLBCL in 1 (11%). Seven (78%) pts had bone marrow involvement. Four pts(44%) developed LON after first line therapy that was RCHOP in 3 (33%) and RFC in 1 (11%); 5 (55%) pts, in relapse, had received previous chemotherapy (containing fludarabine in three cases, 60%) when LON occurred. **Results.** LON appeared after a median of 103 days (range 40-140) from the last Rituximab infusion and median duration was 57 (14-236) days. When LON occurred 7 (78%) pts were EBV positive with high IgG levels, while no viral reactivation or autoimmune disorder was documented. Bone marrow examination revealed hypocellularity and maturation arrest of the myeloid series in 7 (78%) cases and normal cellularity in 2 (23%). Four pts (44%) had decreased immunoglobulin concentrations. Only 2 (23%) episodes of pulmonary pseudomonas infection were documented during the neutropenia period. Three pts (33%) had fever. All pts received corticosteroids and5 pts(55%) had GCSF treatment, but the clinical course of LON was not affected by these treatments, being always self-limiting with complete resolution of marrow impairment. **Conclusions.** In our experience an indolent histology, bone marrow involvement and high EBV IgG levels seem to be related to the onset of delayed neutropenia. A transitory myelosuppressive effect of Rituximab could have a role in the pathogenesis of LON in our cohort of pts. The treatment of LON is not well defined. A watchful waiting approach is appropriate in the majority of patients. Further biological and prospective studies are needed to better clarify the pathogenesis, clinical impact and treatment of LON.

1710

THERAPY FOR THE PATIENTS WITH DIFFUSE B-CELLS LYMPHOMA(DBCL) ASSOCIATED WITH CHRONIC HEPATITIS C (HCV)

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The relationship between lymphoproliferative disorders and infectious agents has been studied for many decades. Epidemiological studies have linked hepatitis C virus (HCV) infection to B-cell non Hodgkin's Lymphomas (B-NHL). According the data of WHO classification in 2008 HCV infection takes part in etiopathogenesis diffuse B-cells lymphomas and those tumors may be virus associated. In a large pooled analysis of combined data from several countries, demonstrated that presence of HCV infection was linked not only to marginal zone lymphoma, considered an indolent course B-NHL, but also to diffuse large B-cell lymphoma (DLBCL), a high-grade B-NHLIn CRC by N. N Blokhina in the period 2000-2011 we observed 556 patients with NHL with diagnosed lymphoma were also surveyed of chronic viral infection. DBCL has been diagnosed in 296 patient, of them in 88 patients HCV was determined by polymerase chain method in blood. Serum level HCV RNA was from negative to 5x10^5 c/ml. Of those 88 patients in 24 patients the disease was diagnosed in I-II stage, in 64 - в III-IV stage. The age of the patients varied from 16 y. o. to 76 y. o, the median was 47 y. o. The correlation between male and female was 2:1. Before therapy 57 patients had ALT to be over normal. Mediana was 3. 1 normal. Among those 88 pts in 56 pts have GCB immunohistochemistry type and 32 pts - was non-GCB type. All 88 patients were treated by immunochemotherapy R-CHOP. In pts GCB group complete remission(CR) was reached in 31(56%) pts, 15(27%) developed partial remission(PR) and 10 pts without effect. Median follow up CR was 28 months, PR- 14months. In pts non-GCB group complete remission(CR) was reached in 15(47%) pts, 10(31%) developed partial remission(PR) and 7 pts without effect. Median follow up CR was 14 months,PR-5 months. During immunochemotherapy serum level HCV RNA has increased at 51 of 88 pts. Median increased level RNA and ALT was after 4. 5 course R-CHOP. Level ALT was from 2 to 50 norm, median- 7. 5 norms. The treatment was stopped at 13 pts because of hepatic toxicity. **Conclusions.** Without initial liver dysfunction, HCV-infected pts with DLBCL can experience a similar outcome compared to their HCV-negative counterparts when treated with standard chemotherapy/immunotherapy despite differences in the presentation of the disease. A significant proportion of patients with HCV + DLBCL develop liver toxicity often leading to interruption of treatment.

1711

IS INTENSIVE CHEMOTHERAPY ASSOCIATED WITH SUPERIOR OUTCOME IN PERIPHERAL T-CELL LYMPHOMA NOS AND ALK-VE ANAPLASTIC LARGE CELL LYMPHOMA?

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Background. The mature T cell lymphomas are an uncommon and heterogeneous group of disease entities. They account for approximately 10% of all lymphoid malignancy and generally carry a poor prognosis. Peripheral T cell lymphoma not otherwise specified (PTCLNOS) is the commonest form of mature T cell lymphoma, accounting for approximately half of all cases. A number of studies have attempted to evaluate the value of treatment intensification, but no firm conclusion has been reached. For over a decade our centre has favoured an aggressive approach for newly diagnosed patients with PTCLNOS, usually consisting of several cycles of salvage type intensive chemotherapy followed by high-dose chemotherapy (BEAM) with autologous stem cell rescue delivered in first response, if the patient is deemed fit enough. A similar approach is adopted for patients presenting with anaplastic large cell lymphoma, ALK-1 negative (ALCL). **Aim.** To determine whether intensive chemotherapy is associated with superior outcome in peripheral T-cell lymphoma (not otherwise specified) and ALK1 negative anaplastic large cell lymphoma. **Methods.** Laboratory records, multidisciplinary team meeting notes and local cancer audit records were searched to identify cases between 1999 and 2011. Where available, case notes were reviewed to confirm eligibility, and gather clinicopathological data and information on clinical outcome and treatment toxicity. Survival analyses were performed using Kaplan-Meier curves and compared using log-rank testing. **Results.** Data was available on 31

patients, of whom 29 had received treatment. 11/ 29 (37. 9%) patients were treated intensively. 12/21 patients with PTCLNOS had a response to treatment (CR or PR), while 5/8 patients with ALCL responded. The overall response rate was 58. 6% (17/29). The median duration of follow-up was 11 months (range 0. 1 to 168 months). There was a trend towards improved overall survival in patients receiving intensive chemotherapy compared with non-intensive (median 49 months vs 6 months), however this was not statistically significant (Figure 1). A trend towards longer progression-free survival in patients achieving a response was also seen with intensive treatment. The international prognostic index was strongly predictive of overall survival (median survival 57 vs 3 months, p<0. 0001). Patients with limited stage disease had significantly longer survival than those with advanced stage disease (57 vs 5 months, p<0. 05). Similarly, elevated LDH, bone marrow involvement and the presence of B symptoms were all predictive of shorter overall survival. There was no significant difference in survival between patients diagnosed with ALCL and PTCLNOS. **Summary.** In this small, single-centre, retrospective study we do not show any significant differences in clinical outcome in patients with PTCLNOS or ALCL with relation to treatment intensity. We chose to exclude all other forms of T-cell lymphoma except PTCLNOS and ALK1-negative ALCL to achieve homogeneity. Nevertheless, the low numbers of patients identified limit the power of this study and this has been a common theme in previous studies. Furthermore, the retrospective approach clearly introduces bias. We intend to expand this study nationally in order to increase statistical power, but there remains insufficient evidence to conclude that intensive treatment is superior in this setting.

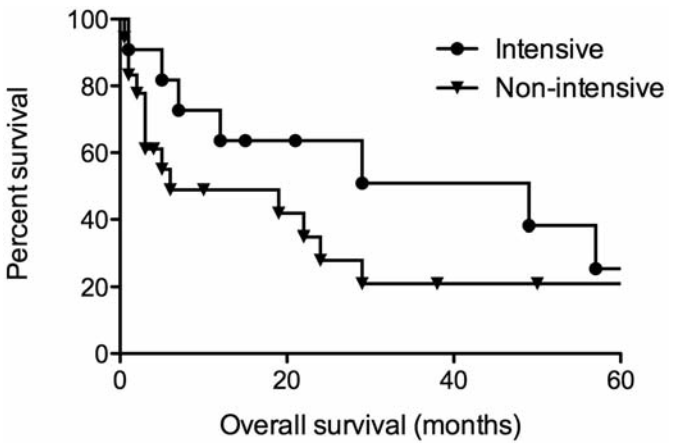

Figure 1. Overall survival by treatment intensity.

1712

CD5-POSITIVE SPLENIC MARGINAL ZONE LYMPHOMA

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Background. Splenic marginal zone lymphoma (SMZL) is a well recognized B-cell neoplasm which characterized by splenomegaly, bone marrow involvement, immunologically by typical phenotype of marginal zone cells. In rare cases there were CD5-positive variants of disease. **Aims.** The aim of our study was to investigate the characteristics of a CD5-positive SMZL. **Materials and Methods.** The study included 6 patients (5 men and 1 woman), who were observed in Hematological ResearchCenter (Moscow,Russia), from January 2000 to February2012. In all cases the diagnosis of SMZL was based on immunophenotyping of peripheral blood lymphocytes and hystochemistry of bone marrow. Immunophenotypic analysis has shown the expression of mature B-cells antigens (CD19, CD20, CD22, FMC7, sIg) and absence of CD10, CD23, CD43, CyclinD1. In all cases, there was coexpression of antigens CD5/CD19. The average age of patients was 61. 5 years (range 46 to 86 years). At the time of diagnosis were recorded B-symptoms, massive or giant splenomegaly. In neither case is not marked peripheral and visceral lymphadenopathy. M-component is not detected in any case. In the peripheral blood of all patients had varying degrees of severity of the three sprout cytopenia. Histological examination of bone marrow revealed involvement by lymphoma. Given the coexpression of antigens CD5/CD19, with the differential diagnosis of cytogenetic study carried out by FISH to detect the translocation t(11;14)(q13;q32). Also there was performed a molecular study by PCR to identify overexpression of CyclinD1. In neither case was not detected t(11;14)(q13;q32) and overexpression of CyclinD1. All patients were given abdominal discomfort and cytopenic syndrome performed splenectomy. **Results.** After splenectomy, all patients had marked clinical and hematological stabilization, which manifested as the disappearance of B symptoms, normalization of peripheral blood count and LDH. All patients are alive. Mean follow-up 68. 7 months. (32-112 months). **Conclusions.** Classically SMZL characterized by the absence of expression of antigen CD5. CD5-positive lymphomas in the literature respond poorly to various treatments and the expression of CD5 antigen is considered as a marker of aggressive disease course. In our study, because of the small number of patients on the prognostic significance of the findings are not possible, though, after splenectomy, all patients had long-term clinical and hematological improvement.

1713

FEASIBILITY OF ANTHRACYCLINE BASED TREATMENT IN UNSELECTED ELDERLY DLBCL PATIENTS

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Background. Diffuse large B cell lymphoma (DLBCL) is one of the most common Non-Hodgkin Lymphoma (NHL) and comprises about 20% of all newly diagnosed lymphoid neoplasms. The mean age at diagnosis is 65 years in the Caucasian population and therefore a substantial proportion of patients comprises elderly people > 75 years. Nevertheless, this population is underrepresented in almost all clinical trials of DLBCL and treatment related toxicity mainly due to anthracycline treatment is a major concern. New regimens or attenuated schedules of anthracyline based chemotherapy may be an option in elderly patients. However, substantial evidence is available that anthracyclines are of significant importance in the treatment. Therefore, several concepts e. g. Charlson index of comorbidity (CI) are under investigation to define factors guiding the management of elderly DLBCL patients. **Methods.** We retrospectively analyzed and characterized 83 consecutively diagnosed patients > 75 years with aggressive B-cell lymphoma (78 cases of DLBCL and 5 cases of follicular lymphoma grade 3) from January 2003 until January 2012 treated at our tertiary cancer center by chart-based review. **Results.** 84% of the patients were treated with an anthracycline, 88% were treated with rituximab and overall 82% of the patients received a combination of anthracycline and rituximab based therapy. The median overall survival (OS) in all patients regardless of treatment was 43 months. Patients treated with an anthracycline and rituximab had a better median OS than patients not eligible for this combination (54 vs 6 months p< 0. 0001). Patients deemed not eligible for combination therapy were significant older, had a worse ECOG status and a higher CI. The cohort of patients treated with an anthracycline and rituximab had a median age of 79 years and 57% (39/68) were > 80 years and 50% had a CI > 1. In this group there was no significant difference in the median OS in patients <80 or >80 years old (49 vs 54 months). Patients with a serum albumin level > 3,5g/dl had a trend to a better OS (59 vs 27 months p=0. 11), but β2-microglobulin levels < 3mg/l could significantly predict a better OS (69 vs 30 months p=0. 019). 68% of the patients received > 5 cycles of therapy with a median cumulative anthracycline dose of 256 mg/m^2. Since 2007 liposomal doxorubicine was available at our department, 70% of 44 patients eligible for an anthracycline treatment received this new formulation because of suggested lower toxicity. Despite significant older age, a significant worse ECOG status and a trend to a higher CI in patients treated with liposomal doxorubicine there was no difference in the median OS compared to patients receiving conventional doxorubicine. **Conclusions.** Considering the OS data of our unselected cohort we think that all patients with DLBCL should be offered curative immunochemotherapy with the goal of a full dosed anthracycline regimen regardless of age. Only the small proportion of patients with severe comorbidities should be excluded from this approach. The prospective use of comorbidity indices or maybe also markers of pharmacogenetics may better inform our choices in the future.

1714

PRIMARY NON-HODGKIN LYMPHOMA OF THE FEMALE GENITAL TRACT: CLINICAL Results OF A RETROSPECTIVE ANALYSIS OF SINGLE INSTITUTION

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Background. Primary extranodal lymphomas of the female genital tract account only for 1. 5% of all non-Hodgkin lymphomas (NHL). The most common subtype is the diffuse large B-Cell lymphoma (DLBCL) more often affect-

ing uterus. A few series have been reported referring to a single or few cases collected in the review of Lagoo et al. There is no widely accepted consensus on the incidence, diagnostic procedure modality, treatment and prognosis. **Aims.** A retrospective analysis has been performed collecting data on patients with female genital tract NHL, to verify the incidence, response to treatment and overall survival. **Methods.** The study population consisted of all female patients (22) with NHL of genital tract treated in our Institute during the period 2000-2009. All patients gave written informed consent. Response has been evaluated according to Cheeson criteria 2007. **Results.** Median age was 52 years (31-79). Sixteen patients presented a diagnosis of DLBCL while 6 patients presented an indolent lymphoma: 4 extranodal marginal zone-B cell lymphoma (MZL), and 2 follicular lymphoma. In 12 out of 22 patients (55%) the uterus was involved as primary site or with other genital organs: 10 patients presented DLBCL, and 2 MZL. Thirteen patients presented Ann Arbor stage I-II (5 presented bulky disease), while 9 presented stage III-IV (3 patients with bulky disease). Five patients presented B symptoms at the moment of diagnosis: night sweats in all cases and, in one case also weight loss. Five patients presented also pelvic pain and/or bleeding. Patients with DLBCL any site received R-CHOP or R-CHOP like regimen for a maximum of 6 cycles; 14 out of 16 patients achieved CR. Two patients relapsed and died for progression disease. Two additional patients in PR received a second line of chemotherapy (including autologous transplantation) obtained a persistent CR. With a median follow-up of 3 years (0. 9-15. 34) 87% of patients are alive and disease free with a median PFS and OS not reached. Within the 6 patients with low grade lymphoma (4 MZL and 2 follicular) 4 were treated as first line with chlorambucil (plus Rituximab in two cases), 1 patient with R-CHOP and the other one with R-CEOP regimen. Five out of 6 patients obtained a CR and only one patient a stable disease with an ORR of 83%. **Conclusions.** Our retrospective study confirms that the most frequent histology of primary extranodal lymphoma of the female genital tract is the DLBCL and the most frequent organ involved is the uterus. However, the incidence of MZL is not negligible: in our experience they represent approximately 20% of all malignant lymphoma. Outcome of patients with high grade lymphoma of female genital tract receiving antracycline containing regimen in combination with rituximab seems to be very favorable since about 80% of them is still alive and free of disease with a median of three years of follow-up. A large retrospective analysis on female genital tract lymphoma is ongoing on behalf of IELSG with the aim to collect a large number of patient providing sufficient information on clinical behavior on such uncommon disease.

1715

RITUXIMAB AS MAINTENANCE TREATMENT IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMAS (DLBCL). A SINGLE CENTER EXPERIENCE

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Background. The addition of Rituximab in CHOP like first line treatment in DLBCL has significantly improved the progression free survival and in some studies the overall survival of patients of any age. The role of Rituximab as a maintenance treatment in aggressive lymphomas after the achievement of complete response remains under investigation. **Aims.** The aim of our study was the assessment of the role of maintenance treatment with Rituximab in patients with aggressive lymphomas after the achievement of complete response with R-CHOP like regimens. **Methods.** Fifty patients (28 male, 22 female, median age 69, range 17-88) with aggressive B cell lymphomas (45 patients with DLBCL,2 pts with primary mediastinal lymphoma, 2 patients with mantle cell lymphoma and 1 with follicular grade 3) received maintenance therapy with Rituximab after the completion of chemotherapy and the achievement of complete remission. The lymphoma was nodal in 29 cases and extranodal in 21 cases. The IPI was low in29 pts, low-inter in14 pts, inter-high in6 pts and high in 1 patient. Adjuvant radiotherapy was given to9 pts, mainly to extranodal sites of lymphoma involvement. 1st line treatment was R-CHOP (median number of cycles 6, range 3-6) in 46 patients, R-CVP in3 pts and MACOP-B in 2 patients with primary mediastinal lymphoma. The vast majority of the patients received maintenance with Rituximab (375 mg/m2 every 3 months for one year) with median time of initiation 1. 8 months after confirmation of remission. The median number of Rituximab cycles was 4 (range 2-8). **Results.** After a follow up of 34 months (range 30-38 mo) 49 out of 50 patients are alive. The 3-year probability of survival is 98%. Eight patients relapsed (7 within the first year after remission). The probability of relapse is 22. 7% for the whole group of patients. Factors that affect the probability of relapse in multivariate analysis were the histologic type (DLBCL vs others) and the serum albumin while hemoglobin and b2 microglobulin were of marginal significance. Age, sex, IPI, performance status, serum LDH, and nodal or extranodal involvement were not significant parameters in univariate and multivariate analysis. **Conclusions.** Our cohort of patients with aggressive lymphomas who received maintenance therapy with Rituximab after remission showed marginally increase in progression free survival in comparison with previous studies. It is apparent that well organized randomized studies are needed in order to clarify the advantage of maintenance therapy with Rituximab in patients with aggressive lymphomas who receive R-CHOP like regimen as standard 1st line therapy.

1716

EFECTIVITY AND SAFETY OF BENDAMUSTINE-RITUXIMAB IN AGED PATIENTS WITH RELAPSED OR REFRACTORY LYMPHOMA

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Background. Bendamustine is a drug with alkylating and antimetabolite properties, with proved activity in relapsed or refractory indolent lymphomas in association with Rituximab. Because of its only moderate toxicity can be safely applied in elderly patients. **Aims.** We present our experience on treating relapsed or refractory low-grade lymphomas and mantle cell lymphomas with the combination of bendamustine and rituximab (BR). **Patients and Methods.** Prospective and observational study of all consecutive patients with relapsed or refractory low-grade lymphoma or mantle cell lymphoma treated with BR since April the 24th 2008 to January 31st 2012. Informed consent was obtained in every patient. Modified Cheson criteria (2007) were used to assess response. Adverse effects were classified using the WHO toxicity criteria. Bendamustine (90mg/m2 daily) was administered the first and second days of each cycle. Rituximab (375mg/m2) was administered a week before the first cycle, the first day of every cycle and four weeks after the last one. Cycles were administered every four weeks to a maximum of six. Patients were evaluable for response if they have received at least two cycles of BR. **Statistical Methods.** Student t, Fisher exact test, Kaplan-Meier tables, log-rank test, Cox multivariate binary logistic and proportional hazard regression. **Results.** Twenty patients were included, 10 females. Mean age: 70 years (44-85); 55% of them were older than 70 years. Diagnosis was follicular lymphoma (FL) in twelve patients, mantle cell lymphoma (MCL) in three, small cell lymphocytic lymphoma (SCLL) in two, lymphoplasmacytic lymphoma (LPL) in two and MALT lymphoma in one. Seventeen patients had relapsed disease and three were refractory to previous treatment schedules. Mean time from diagnosis was 5,5 years (2,6-13,3). Mean number of previous therapies was 3 (1-5), and two patients (one FL and one MCL) have undergone an ASCT. The median number of cycles administered was 4 (mean 4,3; 1-6). Complete response (CR) was achieved in 14 patients (70%) and partial response in 2 (10%). Three patients (15%) showed no response and one was not assessable. None of prognostic variables analyzed (age, sex, functional status, lymphoma type, number of previous treatment and time from diagnosis) was significantly associated with response or toxicity. The median time to treatment failure (TTF) was 19,2months. Median survival (SV) from the BR treatment onset was 25 months (median observation of live patient: 14,4 months). We analyzed prognostic variables in relation to TTF and SV and none was significant. Adverse events were grade 3 or 4 neutropenia in seven patients (35%) and grade 3 or 4 thrombocytopenia in four (20%). An episode of febrile neutropenia and another of coagulase negative staphylococcus bacteriemia associated with indwelling catheter were reported. There was one hepatitis C reactivation. **Summary and Conclusions.** Treatment with BR was effective in a high percentage of our patients with relapsed or refractory low-grade lymphoma. Of note, tolerability and safety were good and the probalility of response and its durability was independent of age. This results confirm the effectiveness of B/R in elderly patients

1717

SURVIVAL AND CLINICAL CHARACTERISTICS OF CHILDHOOD ANAPLASTIC LARGE CELL LYMPHOMA, SINGLE CENTRE EXPERIENCE

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Background. Anaplastic large cell lymphoma (ALCL) is a rare disease in children accounting for 10% to 15% of all childhood non-Hodgkin lymphomas

whose diagnosis and treatment have largely evolved since its initial description in 1985. ALCL is characterized by the proliferation of large pleiomorphic cells of a T/null phenotype that tend to invade the lymph node sinuses and express the Ki1/CD30 antigen. **Aims.** Aim of this study is to report the clinical manifestation and therapy outcome in a cohort of children diagnosed with ALCL and treated in our Department. **Patients and Methods.** We retrospectively reviewed our experience in the field, and report our single institution experience in a series of 8 children diagnosed with ALCL and treated in our Department from January 1995 until December 2010. **Results.** The median age was 6 (range 4-12), 7 male and 1 female, including two children with null phenotype. Lymph nodes were involved in 57% (4/7) of patients. Extranodal disease was found in 43% of the cases, including visceral involvement in 57% (4/7) and bone lesions in 14%. Bone marrow and CNS disease were not encountered. ALK abnormalities were evaluated in 3 patients by assay for the t(2;5)(p23;q35) translocation by RT-PCR and were all found positive. One patient (6%) was stage I by St Jude classification, 3 patients stage II and 4 stage III. Therapeutic strategy included short pulse NHL-BFM90 in 2 patients and HN 97 (MRC) in 5 patients. The patient with bone lesion received chemotherapy according to HN97 and local irradiation, and autologous stem cell transplantation. All patients are alive and disease-free. The median follow-up of the whole population is 10. 7 years and the maximum 14 years. **Conclusions.** Although the optimal treatment is still unknown, current treatment regimens are highly efficacious. Nevertheless, in the future treatment strategies should focus on the refinement of the balance between treatment burden and individual patient risk for failure.

1718

CHARACTERISTICS OF THE PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME COMPLICATED WITH LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Objective. The present study was aimed to improve the understanding of diagnosis and treatment of patients with primary Sjögren's syndrome (pSS) complicated with lymphoma by investigating the clinical characteristics,treatment and prognosis. **Methods.** The data of clinical features, laboratory findings, therapeutic response and follow-up of patients with primary Sjögren's syndrome complicated with lymphoma from January 2006 to January 2011 in our single center were retrospectively analyzed. **Results.** Totally twelve inpatients with pSS complicated with lymphoma were diagnosed, who accounted for 1. 29% of newly-diagnosed lymphoma inpatients during the same period. The main pSS manifestations were xerostomia (n=12), xerophthalmia (n=12) and parotidomegaly(n=6). The characteristic serologic changes were hyperimmunoglobulinemia (n=10), hypocomplementemia (n=7) and decrease of CD_4 T cell number (n=4). The main lymphoma manifestations were lymphadenectasis (n=10) and splenomegaly (n=6). In our study, non-Hodgkin's lymphoma (NHL) was the most common type, accounting for about 91. 7% (11/12) and Hodgkin's lymphoma (HL) accounted for about 8. 3% (1/12). The main pathological subtype was diffuse large B cell lymphoma (DLBCL) (n=5). Extranodal involvement (ten patients) was common, most frequently in the livers (n=3) and the lungs (n=3). About 83. 3% (10/12) patients were in advanced stages, Ann Arbor stage III-IV, at diagnosis. All patients received treatment with combination chemotherapy regimens. Most of the NHL patients received CHOP-like regimens, and the HL patient received AVD regimen. After a median follow-up of 22 months (range 2-54 months), overall survival was 9/10. Except two patients were lost follow-up, two patients was in CR (complete response), four were in PR (partial response), three were in PD (progressive disease) and one patient died from infection during the treatment. Correlation between serum complement level and prognosis of 12 patients with pSS complicated with lymphoma showed that lower C3 and C4 levels presented with poor prognosis. **Conclusions.** The patients with pSS complicated with lymphoma were not rare clinically. It was characterized for these patients by typical serological changes, such as hyperimmunoglobulinemia, hypocomplementemia and decrease of CD_4 T cell number, NHL as the major pathological type, higher frequency of extranodal involvement, advanced stages at diagnosis and expected unfavorable prognosis.

1719

A COLLABORATIVE NATIONWIDE LYMPHOMA STUDY IN LEBANON: INCIDENCE OF VARIOUS SUBTYPES AND ANALYSIS OF ASSOCIATIONS WITH VIRUSES

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Background. Incidence of various Hodgkin (HL) and non-Hodgkin lymphoma (NHL) subtypes and association with viruses in Lebanon are not known. **Aims.** We have conducted a prospective study that assessed the incidence of lymphoma subtypes and evaluated possible associations with various viruses in adult patients with newly diagnosed lymphomas in Lebanon. **Methods.** This is a nationwide study that included 272 adult patients (138 males, 134 females; ≥18 years) diagnosed with various subtypes of lymphoma in Lebanon in 2007. Clinical and pathology data was collected in a data sheet form which comprised available pathology material for each subject which was reviewed according to the 2001 World Health Organization (WHO) classification system by a panel of expert pathologists. Peripheral blood was withdrawn for serology testing for the following viruses: HCV, HIV, EBV, and HTLV-I. **Results.** HL comprised 32. 7% (n=89) of cases while NHL represented 67. 3% (n=183). Consistent with the published literature, nodular sclerosis was the most predominant HL subtype (n=57/89). Among NHL, B-cell NHL represented 88% (n=161/183) while T-cell NHL represented 9% (n=17/183) whereas in 2. 7% (n=5/183) it was not classifiable. The B-cell NHL comprised predominantly diffuse large B cell lymphoma (74 cases; 46%), and follicular lymphoma (37 cases; 23%) (Figure 1).

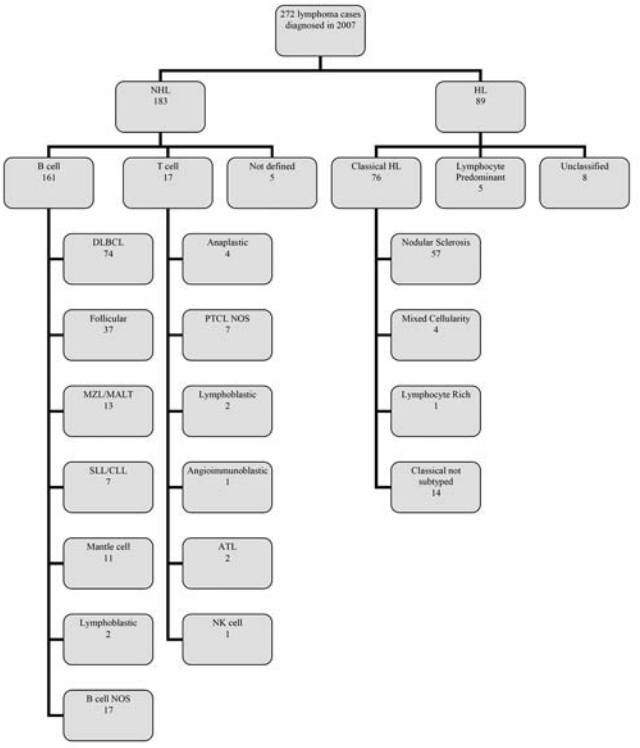

Figure 1. Distribution of the 272 lymphoma cases by subtypes.

Pathology review of 81 cases showed 87. 6% concordance rate. Serology was negative for HCV in 122 tested cases. HIV was positive in 2 cases (1 NHL, 1 HL). Two adult T cell leukemia/lymphoma were HTLV-I positive. EBV IgG were positive in 108/122 (88. 5%) cases (69/79 NHL, 39/43 HL). Also, 38 EBV seropositive cases [27 NHL (24 B-cell type and 3 T-cell type) and 11 HL] were studied for EBV genome expression using EBV-encoded RNA (EBER)-*in situ* hybridization. EBER expression was positive in 8 (21%) cases, of which 6 were HL and 2 were T-cell NHL. **Conclusions.** The distribution of lymphoma subtypes in Lebanon appears similar to that of Western countries. The observed diagnostic concordance rate of 87. 6% is particularly noteworthy and provides a sense of assurance regarding the quality of pathological readings. The high rate of EBV positivity in HL and T-cell lymphoma by EBER is noteworthy and deserves further investigation.

1720

PROGNOSTIC IMPACT OF SERUM VITAMIN D CONCENTRATION LEVEL IN PATIENTS WITH LYMPHOID MALIGNANCIES

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The incidence of lymphoid malignancies has been increasing rapidly. Despite growing evidence for a relationship between serum vitamin D level and solid tumor risk, far less is known about the relationship between vitamin D and the risk of hematologic malignancy. This study aimed to assess the prognostic relevance of serum Vit D level in patients with certain types of lymphoid malignancies. The study was carried out on 75 B-CLL and 120 NHL patients as well as 10 normal healthy controls. For all patients and normal controls serum Vit D were assayed by ELISA. Serum Vit D concentrations level was significantly decreased in both B-CLL and NHL patients as compared to matched controls (P =0. 000*). In B-CLL patients serum Vit concentrations level was significantly negatively correlated with other prognostic factors (CD 38%) (r = -0. 630, P = 0. 001*), ZAP 70% (r = -0. 603, P= 0. 001) and Binet staging (r = -0. 874, P = 0. 000). More over, in NHL patients serum Vit D concentrations was significantly negatively correlated with performance status (r = -0. 373, P= 0. 04), IPI (r = -0. 413, P = 0. 02) but insignificantly correlated with staging (r=-0. 281, P = 0. 132). B-CLL and NHL patients with seriously deficient serum Vit D level concentrations had significantly shortened life span (P =. 0000*). So, serious Vit D insufficiency represents significant poor prognostic factor in B-CLL and NHL patients.

1721

WITHDRAWN

1722

EPIDEMIOLOGICAL DATA OF VENEZUELAN PATIENTS WITH LYMPHOMA. IMPROVEMENT OF THE OUTCOME OF FOLLICULAR LYMPHOMA (FL) AND DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) WITH R-CHOP VS CHOP

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Background. Lymphomas are among the most frequent hematological neoplasias in Venezuela. **Aims.** To evaluate epidemiological data of lymphoma patients treated at the Oncology-Hematology Institute and other Public and Private Clinics in Caracas, Venezuela, during the last 14 years and to compare the efficacy of the treatment R-CHOP vs CHOP in FL and DLBCL. **Methods.** Diagnosis was established by lymph node biopsies, histopathology and immunohistochemistry, complemented with X rays and TACs. **Results.** Out of 1812 lymphoma patients, 1214 were diagnosed as non-Hodgkin lymphoma (NHL, 65. 1%), 598 as Hodgkin Lymphoma (HL, 34. 9%). Subtypes of HL: Nodular Sclerosis (244; 129 female, 115 male; average age 32. 4 years-old (18-65), Mixed Cellularity (109%; 37 female, 72 male; average age 22. 3 years-old (18-56), Lymphocyte Predominance (17; 6 female, 11 male; average age 22. 6 years-old (18-67), Lymphocyte Depletion (21; 6 female, 15 male; average age, 22 years-old (20-42). Staging of HL patients: I: 5. 5%, II: 44%, III: 31. 1%, IV: 19. 4%. They were treated with different protocols (MOPP, ABVD, STANDFORD V, HYBRIDO, COPP/ABVD, COPP/EBVD) according to the year treatment was initiated, and since 2004 with BEACOPP and in some cases radiotherapy. Subtypes of NHL: DLBCL (442), FL (293), MALT lymphoma (121), Peripheral T cell (7), Mantle Cell lymphoma (4); miscelaneous (mycosis fungoides, immunoblastic, anaplastic, Lymphoblastic, Burkitt or Cutaneous B cell lymphoma) (248). Of the FL 45% had high FLIPP, while 60% of the DLBCL had intermediate high IPI score with high-risk prognostic factors; only 15% of cases had low risk lymphoma. They were treated with different protocols according to the year of diagnosis: CHOP, CHOP Bleo, MACOB B, ATT, HyperCVAD, and CHOP MTX and since 2004 with R-CHOP. 29 patients were treated with CHOP, 55 patients with R-CHOP. The R-CHOP as induction in FL *de novo* and Rituximab as maintenance produced 46 % CR and 56% PR. The CR increased to 77% after two years of Rituximab maintenance during four-year observation. Global survival in FL was 89. 5% for R-CHOP vs. 50% for CHOP; the survival free of events in FL was 89. 8 for R-CHOP vs. 39. 1% for CHOP. The CR for DLBC lymphoma was 68%. The global survival in DLBCL was 92. 1% for R-CHOP vs. 67. 9% for CHOP and the survival free of events in DLBCL was 90% for R-CHOP vs. 44% for CHOP (p = 0,002). The patient age influenced the survival while other variables (sex, type of lymphoma or stage) did not have impact in survival free of events period. An improvement in the outcome of lymphoma patients occurred when the patient was treated with R-CHOP. **Conclusions.** The most frequent HL subtype was nodular sclerosis (62. 6%), mainly women and young adults; Lymphocyte Predominance and Lymphocyte Depletion were only 4. 3 % y 5. 4%. The most frequent NHL subtype was DLBCL, followed by FL. The distribution of the subtypes of lymphoma in Venezuela follows the same pattern reported in other populations. The global survival and the survival free of events were improved with R-CHOP. At this time this must be the treatment of choice.

1723

THE ROLE OF REGULAR CT IN FOLLOW-UP AFTER THE TREATMENT OF LYMPHOMAS

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Background. Despite annual improvement of therapy results of lymphoma patients certain group of these patients will have relapse of the disease. There is no definite opinion should the regular CT be done in the follow-up (FU) period for the detection of relapse. **Aims.** The aim of the study was evaluation of the role of regular CT in the FU period after the completion of the treatment of lymphomas.

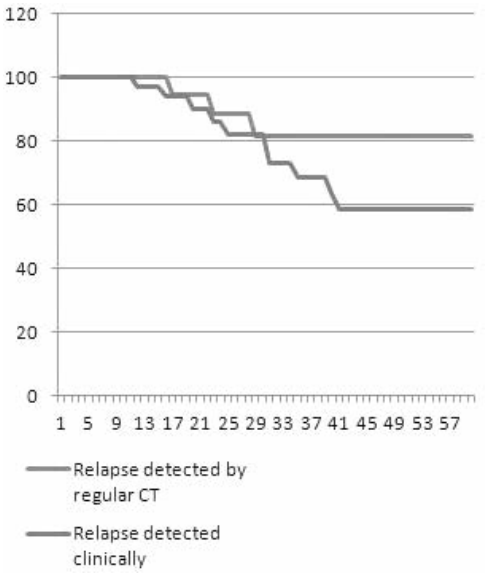

Figure 1. 5-year OS rate in the groups of patients in which relapse was detected by regular CT and clinically.

Methods. In the period of 2008-2011 years 58 patients with Hodgkin's Lymphoma (HL) (22 patients) and Non- Hodgkin's Lymphoma (NHL) (36 patients) were treated in the relapse of the disease diagnosed at least 4 months after

the end of the initial treatment FU period was 9-196 months (median 34 months) in the whole group of patients, 14-89 months (median 31 months) - in the group of NHL patients and 9-196 months (median 46 months) - in the group of HL patients. The FU strategy was based on the regular visits to the physician every 3-12 months (patient complaints, disease history were evaluated, physical examination and blood tests were performed) and CT performing every 6-12 months corresponding to the period of observation. **Results.** Relapses were detected in the period 4-190 months after the completion of the initial treatment (median 13 months). In the group of HL they were detected in the period 4-190 months (median 27 months), in the group of NHL - 4-69 months (median 12 months). 62% of relapses were assumed clinically, 64% - in HL group and 61% - in NHL group. In the group of indolent lymphomas 58% of relapses were assumed clinically, in the group of aggressive lymphomas - 65% (p>0. 05). Significant difference was detected in the overall survival (OS) in the group of patients in which relapse was detected by CT compared to the group of patients in which relapse was detected clinically. Median survival wasn't reach in both groups. 5-year OS was 58. 6±9. 9% in the group of patients in which relapse was assumed clinically and 81. 8±9. 2% in the group of patients with CT assumption of relapse (p<0. 05). **Conclusions.** Symptoms of relapse and clinical examination of patients play the most common role in the assumption of the relapse in patients with lymphomas even in case of regular CT examination. However this study revealed the benefit in the OS in the group of patients in which relapse was detected by regular CT. More precise role of this diagnostic method should be evaluated in large prospective studies.

1724

HIGH INCIDENCE OF LATE DEATHS AFTER RITUXIMAB COMBINED CHEMOTHERAPY: A 20-YEAR RETROSPECTIVE FOLLOW-UP STUDY IN PATIENTS WITH DLBL

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Background and Aims. In Japan, rituximab was generally used for patients with diffuse large B cell lymphoma (DLBL) after 2003 (rituximab era), and rituximab combined Chemotherapy has greatly improved outcomes. We treated 121 patients (69 men and 52 women, median age 68 years, range: 19 to 93 years) with DLBL in 20 years (1990-2009), and analyzed their prognosis retrospectively to overall survival curve (OS) at single institute. **Patients and Methods.** 121 patients were treated in 20 years. 56 patients (34 men and 22 women, median age 67 years, range: 37 to 93 years) were treated without rituximab (CHOP-like regimen 48 patients and other therapy 8 patients in pre-rituximab era), 65 patients (36 men and 29 women, median age 69 years, range: 19 to 86 years) were treated with rituximab in rituximab era (R-CHOP regimen 62 patients, other chemotherapy 3 patients). The over-all survival curve of 120 patients with DLBL was analyzed using the log-rank test and expressed as Kaplan-Meier plots.

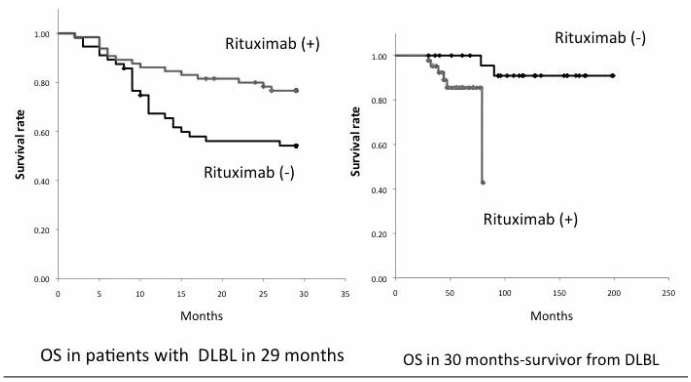

Figure 1. OS in patients with DLBL.

Results. In 29 months after diagnosis, overall survival curve was significantly (P<0. 05) prolonged rituximab treated patients (29 months over-all survival rate is 76. 9%) than patients without rituximab therapy (29 months over-all survival rate is 55. 4%). 28 patients (17 men and 11 women, median age 62 years, range: 41 to 77 years) without rituximab therapy and 43 (24 men and 19 women, median age 68 years, range: 19 to 85 years) patients with rituximab therapy have been followed up after 30 months. We analyzed for overall survival curve limited to survivor from DLBL after 30 month later. From 30 months later after diagnosis, 6 rituximab treated patients died (3 patients died due to secondary malignancy, and 3 patients died due to DLBL relapse), and only 2 patients without rituximab therapy were died (one patient died due to secondary malignancy and one patient died due to haemolytic anaemia). In over 30 month-survivor from DLBL, the over-all-survival curve of survivor with rituximab (72 month over-all survival rate is 88. 3%) is significantly (P<0. 05) shorter than survivor without rituximab (72 month over-all survival rate is 100%). **Conclusions.** Rituximab combined chemotherapy is effective for DLBL. However, it is necessary to long-term follow-up because of increasing late death from over 30 month later after diagnosis.

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FOLLICULAR LYMPHOMA TREATMENT. 10 YEARS FOLLOW UP. ONE CENTER EXPERIENCE

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Background. There are no follicular lymphoma treatment standards. Clinical management of patients ranges from observation without therapy to high-dose chemotherapy followed by autoSCT or alloSCT. Most commonly used chemotherapy programs are R-CVP, R-CHOP and R-F(M)C. **Aims.** Assess of treatment results in the National Research Center for Hematology, Moscow, Russia from 2001 to 2011. PATIENTS AND **Methods.** The results of therapy of 82 patients with follicular lymphoma treated from 2001 to 2011. The analysis included 34 men (41%), 48 women (59%). The mean and median age was 53 years (range: 27-83 years). Patients were divided into groups according to the FLIPI criteria: I risk group - 28 (34%), II - 15 (18%), III - 39 (48%). In 14 cases (17%) it was I histological grade of follicular lymphoma, in 40 (49%) - II, in 28 (34%) - IIIAB. Induction courses were R-CVP, R-CHOP and R-F(M)C. The KaplanMeier method was applied to calculate the overall survival (OS) and event-free survival (EFS). **Results.** Most patients received induction courses with anthracyclines: R-CHOP (n=32) and CHOP (n=9). 13 patients received R-FMC, 3 - CVP, 3 - R-CVP. Non-program courses were made to 15 patients. 7 patients are under observation without treatment (Median (Med) 35 months). In the first relapse patients after R-CVP received R-CHOP, in the second relapse - R-FMC. In the first relapse after R-CHOP patients received R-FMC, and in following relapses - non-program treatment. The 5-year overall survival (OS) after R-CVP, R-CHOP, R-FMC courses was 71%, 63%, 99% respectively (p=0. 05) (Figure 1).

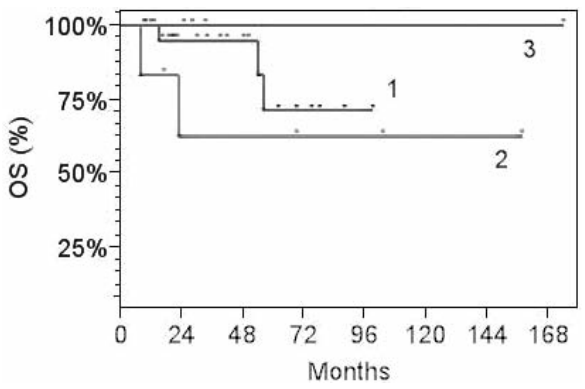

Figure 1. Overall survival (OS) after R-CVP (1), R-CHOP(2), R-FMC(3).

Event-free survival (EFS) during 5-years in R-CVP group was 42% (Med 56 months), R-CHOP - 21% (Med 39 months). 3-years EFS in R-FMC group was 38% (Med 32 months, p=0. 05) (Figure 2).

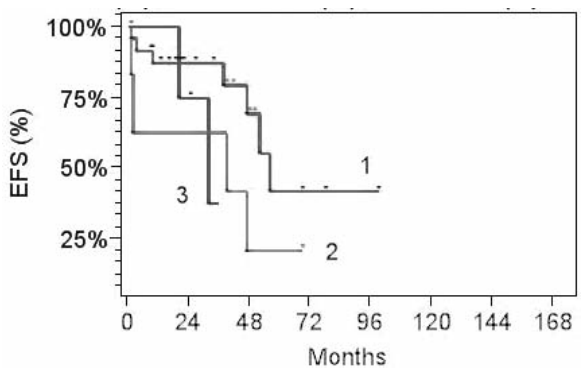

Figure 2. Event-free survival (EFS) after R-CVP (1), R-CHOP(2), R-FMC(3).

Patients with I and II histological types received R-CHOP and R-FMC equally. Anthracycline-based courses were selected for patients with III histological grading. 21 (26%) patients died (Med 24 months, in 80% FLIPI was III-IV). 10 of them died from resistant tumor progression in retroperitoneal space during 4 years from putting the diagnosis (Med 47 months, 5 patients with II grade, 5 patients with IIIAB grade). These 10 patients were 53% of all patients with bulky disease in retroperitoneal space (n=19). The 5-year OS in the group without retroperitoneal bulky disease (first group) was 98% (p = 0,001), but with the bulky disease (second group) - 50% (Med 56 months) (Figure 3).

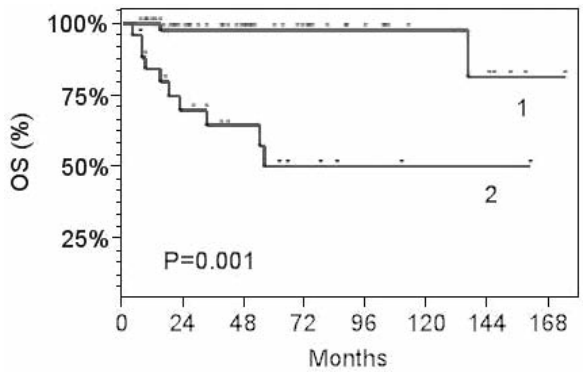

Figure 3. OS in the group without retroperitoneal bulky disease (1), with the bulky disease (2).

Probability of death during the first 5 years in the second group is 10 times higher than in the first group. All deaths in the second group occurred within the first 5 years after treatment (mean time to death is 42 months). The 5-year EFS was 58% (Med 72 months) and 42% (Med 48 months) (p = 0,05) respectively (Figure 4). Probability of relapse and progression is 1,5 times higher than in the first group.

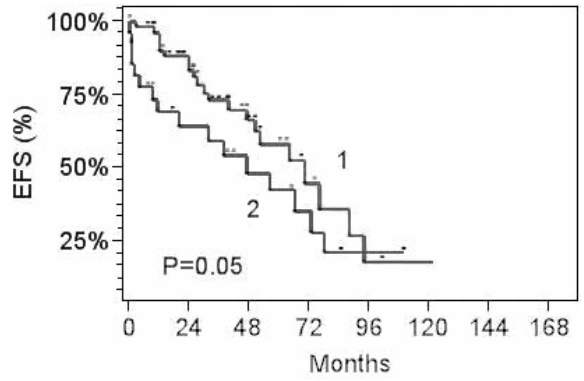

Figure 4. EFS in the group without retroperitoneal bulky disease (1), with the bulky disease (2).

Conclusions. The results indicate that III - IV stage follicular lymphoma with the bulky in the retroperitoneal space is an independent predictor of poor prognosis. Possibly, this group of patients needs early intensification of treatment.

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ORBITAL LOCALIZED MANTLE CELL LYMPHOMA

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Background. Mantle Cell Lymphoma (MCL) is an aggressive lymphoma diagnosed in the majority of the cases in advanced stages. The orbital extra nodal affection is infrequent. **Aims.** To describe the characteristics, evolution, treatment and survival data among the patients diagnosed with orbital MCL. **Materials and Methods.** From August 1992 to December 2010, medical records of patients diagnosed of MCL at the Institut Català d'Oncologia Hospital Duran i Reynals were reviewed. Eighty-six MCL patients were identified, 7 of whom were diagnosed in the orbita. Pathology reports, histological sections and immunohistochemical-stained slides were reviewed. Two cases in which diagnosis was not confirmed were excluded. **Results.** Five (5. 8%) orbital MCL patients were identified, and three of them (3. 5%) had localized disease. The median age of patients with localized disease was 75 yr (39-79). Two of them were women. Clinical presentation consisted in a non bulky unilateral orbital mass. None of the 3 patients had B symptomsThe biopsy showed a lymphoid infiltration with follicular growth pattern in one case and a vaguely nodular and diffuse growth pattern in the other 2 cases. All the cases were positive for CD5 and Cyclin-D1. Sox11 staining has been requested and it is pending. Treatment of these patients consisted on 6 cycles of CHOP chemotherapy in one patient and combined therapy with 3 cycles of CHOP-like chemotherapy and involved-field radiotherapy in the other two. All of them achieved complete remission. With a median follow-up of 35. 3 months, one patient progressed in Waldeyer region and achieved a second complete response after involved field radiotherapy. All of them are alive and disease free. **Conclusions.** Localized orbital MCL is a rare presentation but should be considered in the differential diagnosis of lymphomas in this area. Growth pattern and immunohistochemistry is comparable with nodal MCL. Responses to treatment are good and clinical evolution seems to be more indolent that their nodal counterpart.

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PRIMARY CEREBRAL LYMPHOMA- SINGLE INSTITUTION EXPERIENCE 2004-2011

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Background. Primary Cerebral Lymphoma (PCL) is a rare form of lymphoma. In 2009, the IELSG published results of a randomised control trial demonstrating the benefit survival of adding high dose cytarabine to the standard chemotherapy of high dose methotrexate [HDMX]. However, toxicity was greater following the addition of cytarabine with 8% dying of sepsis and 44% of patients [vs. 2. 5%] requiring a dose reduction. Given the better survival seen in the cytarabine arm it was added to local PCL treatment protocols. Due to concerns over its toxicity, cytarabine was introduced at a 50% dose of that in the published trial. Also, treatment outcomes of PCL have often been hampered by high levels of neuro-cognitive toxicity often attributed to radiotherapy. **Aims.** 1] To assess the outcome of PCL treatment to see if treatment changes in 2009 have improved patient survival and if so, is it comparable with published series given the dosing reductions used. 2] To assess the functional cognitive ability of patients at diagnosis and one year post treatment by assessing ability to live independently. **Methods.** All patients diagnosed with PCL over the past eight years were included in a retrospective review. Data was collected from case notes. **Results.** 26 cases of PCL were identified retrospectively (16 male and 10 female) with an average age of 58. 9 years. 15 patients [58%] received first line chemotherapy, 10 patients [38%] were treated with radiotherapy alone due to poor performance status. One renal transplant recipient had neither modality and was successfully managed with reduction in immunosupression alone. Chemotherapy for patients treated up to 2009 [10 patients] was HDMX alone and for 5 patients treated after 2009 was HDMX with HD Cytarabine. All patients receiving the HDMX and cytarabine post 2009 protocol received the full dose intended with no dose reductions. All cycles were supported with pegylated GCSF administration. Consolidatory radical radiotherapy was delivered following completion of chemotherapy. CR at 3 months post treatment was 92% [24/26 patients] and current survival at 34. 2 months mean follow up is 65% [17/26 patients]. Of the 9 patients who have died, 7 died from progressive disease, one died of pneumonia and one died of an Alzheimer's like illness. Overall survival at 12 months was 70%, at 24 months was 62% and at 36 months was 38%. Patients treated with the addition of high dose cytarabine currently have an average follow up of 28 months and all are in remission. With regards assessment of cognitive function there was an overall improvement in their functional level one year post treatment [61 vs. 50% living independently]. This is contrary to the perceived toxic effects of treatment and likely reflects reduced tumour burden. **Conclusions.** Our outcomes in PCL are comparable to modern published series. It is our view that when appropriate selection criteria are applied that HDMX with HD cytarabine, at a dose level approx 25% below that achieved overall in IELSG trial, is a tolerable and efficacious regimen.

1728

PREVIOUS RITUXIMAB EXPOSURE DOES NOT AFFECT CLINICAL OUTCOMES OF HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM-CELL TRANSPLANTATION IN PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Salvage chemotherapy followed by high-dose therapy and autologous stem-cell transplantation (ASCT) has been the standard treatment for relapsed or refractory diffuse large B-cell lymphoma (DLBCL). With the beginning of rituximab era, the role of rituximab in the salvage treatment is currently under investigation, and in that context, it has become an issue that prior rituximab exposure may affect the efficacy of rituximab in salvage treatment. In addition, the relationship between rituximab exposure in the first-line treatment and the response to salvage treatment including ASCT but without rituximab has not been investigated. **Aims.** In the present study, we investigated the association of previous rituximab exposure with the clinical outcomes of salvage treatment without rituximab and ASCT in patients with relapsed or refractory DLBCL. **Patients and Methods.** Eighty nine patients with relapsed or refractory DLBCL have undergone ASCT in the Asan Medical Center from April 1994 to August 2011. Among them, 19 and 25 patients were treated with CHOP and RCHOP, respectively, as the first line treatment. Event free survival (EFS) and overall survival (OS) were calculated from the start of conditioning regimen to event or deaths. **Results.** Baseline characteristics between the two groups were not significantly different, including age, prior chemotherapy before ASCT, age-adjusted IPI score at the time of ASCT, Ann-arbor stage, the time from diagnosis to relapse or progression (T/rel) and disease status before ASCT (complete response [CR] versus partial response [PR]). ESHAP regimen was the most frequently used salvage regimen in both groups (47% in CHOP and 68% in RCHOP group, p=0. 208). BEAC (63%) and BUCYE (64%) protocols were the most frequently used conditioning regimen in CHOP and RCHOP group, respectively. With a median follow-up duration of 86 months, median EFS and OS were not significantly different (CHOP vs RCHOP, EFS, 4. 1 months vs 6. 7 months, p=0. 636; OS; 8. 0 months vs 10. 4 months, p=0. 635, respectively). Response rates to ASCT which include CR and PR, were 74% and 88% in CHOP and RCHOP groups, respectively (p=0. 459). Only age-adjusted IPI score at the time of ASCT (2 or 3) was the independent prognostic factor affecting OS in both univariate and multivariate analyses (HR 4. 60, p=0. 001 and HR 4. 63, p=0. 002, respectively). Multivariate analysis also showed that prior rituximab exposure did not affect OS (HR 1. 20 95% CI, 0. 57-2. 50, p=0. 636). **Conclusions.** Prior exposure to ritiximab does not seem to affect the clinical outcomes of salvage treatment without rituximab and ASCT treatment in patients with relapsed or refractory DLBCL.

1729

CODOX-M/IVAC AS TREATMENT FOR AGGRESSIVE B-CELL NHL: UNICENTRIC EXPERIENCE

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Objectives: To analyse results of remission, survival and complications related to CODOX-M/IVAC treatment in patients diagnosed of NHL-DLBCL, NHL-DLBCL-Burkitt-like or anaplastic NHL-DLBCL. **Patients and Methods.** Between October 2005 and December 2011, 19 consecutive patients (52. 6% men) were diagnosed in our institution from NHL-DLBCL (n=12, 63. 2%), NHL-DLBCL-Burkitt-like (n=5, 26. 3%) and anaplastic NHL-DLBCL (n=2, 10. 5%). Six patients had previously received another chemotherapy regimen. The international prognostic index (IPI) was: Low (n=4, 21%), intermediate-low (n=4, 21%), intermediate-high (n=8, 42%) and high (n=3, 16%). Ki67 was studied in 17 patients and 11 (65%) showed >90%. C-myc was studied in 10 cases and the rearrangement resulted positive in 3 of them (30%). The median age was 58 years (range:14-76). Twenty-six per cent (n=5) of the patients were over 65 years-old. Intensive chemotherapy regimen administrated to patients under 65 years-old was CODOX-M-R (2 cycles): Rituximab 375mg/m2 (day 1), cyclophosphamide 800mg/m2 (day 1) and 200mg/m2 (days 2,3,4 and 5), adriamicin 40mg/m2 (day 1), vincristine* 1. 5mg/m2 (without maximum. Days 1 and 8) and methotrexate* 1200mg/m2 administrated in 1 hour and 240mg/m2/h in 23 hours and intrathecal cytarabine (days 1 and 3) and intrathecal methotrexate (day 15). IVAC-R (2 cycles): Rituximab 375mg/m2 (day 1), Ifofosfamide* 1500mg/m2 (days 1,2,3,4 and 5), etoposide 60mg/m2 (days 1,2,3,4 and 5), cytarabine* 2gr/m2/12 h. (days 1 and 2) and intrathecal methotrexate (day 5). [* Ajusted for patients >65 years]. **Results.** Fifteen patients (88. 23%) responded to treatment: 87% (n=13) as complete remission (CR) and 13% (n=2) as partial remission (PR). Relapse rate was 11. 7% (n=2). Two patients (10. 5%) died because of sepsis after first CODOX-M. Treatment was interrupted in 2 patients, one because progression and another because neurotoxicity. A microbiological documentation of infection was demonstrated in 89. 5%. Seven patients (36. 8%) suffered from invasive fungal infection (6 oropharingeal candidiasis and 1 mandibular aspergillosis). The rate of severe mucositis (grade 3-4) was 31. 6% (n=6). The days of neutropenia and hospitalization after each cycle are shown in Table 1. Overall survival (OS) was 73%±10% with a median follow-up of 32 months (range:1-60), progression-free survival (PFS) was 77. 8%±15. 2%. Overall survival was higher in patients with high or intermediate-high than in patients with low or intermediate-low IPI (91% vs. 63%) (p=0. 18). Mortality related to was 10. 5% (n=2). **Conclusions.** CODOX-M/IVAC is a good therapeutic option for aggressive B-NHL with a rate of response of 88%, PFS of 78% and an OS of 73%. However, a high rate of infections and long hospitalizations should be considered before selecting patients for this therapy.

Table 1.

	NEUTROPENIA Median (range)	HOSPITALIZATION Median (range)
1st CODOX-M	10 days (1-21)	23 days (17-43)
1st IVAC	6 days (0-11)	18.5 days (8-40)
2nd CODOX-M	13 days (1-34)	28 days (15-48)
2nd IVAC	6 days (0-15)	17.5 days (5-37)

1730

FDG-PET CT SCAN CONTRIBUTION IN DIAGNOSIS AND INITIAL STAGING OF INDOLENT LYMPHOMAS

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Background. FDG PET-CT scan (PET) contributes in the diagnosis and follow-up of patients with lymphoma. Its role is mostly defined in aggressive lymphomas while its use in initial diagnosis of indolent lymphoproliferative diseases is not adequately studied. **Aims.** The study of PET contribution at initial diagnosis and staging of patients with indolent lymphomas who underwent the usual staging with CT and bone marrow biopsy for the evaluation of their disease. **Methods.** Between 2003-2011 patients with indolent lymphomas underwent staging with standard methods (CTs, blood and bone marrow histological, immunophenotypic and molecular studies) as well as PET, at diagnosis of their disease. Patients' characteristics were studied and comparison of findings with standard methods and PET findings was performed. **Results.** From a total of 55 patients who were studied, 22 were diagnosed with follicular lymphoma, 22 with marginal zone lymphoma (7 splenic, 7 primary bone marrow, 5 extranodal, 3 nodal), 6 with SLL, 2 with mantle cell lymphoma and 3 with Castleman disease, NK and T-LGL leukemia, respectively. The disease stage, as shown by standard staging methods was early (I and II) in 22 patients and advanced (III and IV) in 34 patients. In early stage patients, PET uncovered less disease sites in 6 patients and more sites in 4, compared to CT. On the contrary, in advanced stage patients, PET demonstrated less disease sites in 3 and more disease sites in 19 patients, compared to standard CT scan. Mean value of PET uptake in early stages was 5,93±2,73 and in advanced stages 5,73±3, a difference not statistically significant. From 26 patients in whom bone marrow infiltration was documented with standard bone marrow studies, PET was able to trace bone marrow infiltration only in 5 (19%). Moreover, while 18 patients presented splenomegaly in CT, PET disclosed abnormal splenic uptake in 9 of them. Specifically, in patients with splenic marginal zone lymphoma who all had splenomegaly, PET indicated abnormal uptake of the spleen in 71%. Additionally, in all patients with primary bone marrow lymphoma who by definition do not present splenomegaly, PET did not present an abnormal splenic uptake. On the contrary, in 2 patients with follicular lymphoma an abnormal splenic

uptake was seen without evidence of splenomegaly. **Conclusions.** PET can contribute in identification of more disease sites of indolent lymphomas and could be used when stage or sites confirmation is crucial for the selection of the appropriate treatment strategy. It can not be safely used for the diagnosis of bone marrow infiltration and should not substitute standard bone marrow studies in indolent lymphomas. The precise role of PET scan in the identification of spleen infiltration by indolent lymphomas needs further elucidation.

1731

COMPARISON OF RITUXIMAB-CHLORAMBUCIL(R-CHL) COMBINATION WITH OTHER FIRST LINE TREATMENTS IN MANTLE CELL LYMPHOMA (MCL) PATIENTS: EXPERIENCE FROM 6 CENTERS IN GREECE

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Background. MCL is associated with poor prognosis with a median survival of 3-5 years. Treatment approach of MCL is still quite challenging and therapeutic options are variable and controversial. Our Unit has adopted the combination of R-Chl, a completely different therapeutic strategy of low toxicity producing high response rates,as first line treatment in patients with MCL. **Aims.** To compare the R-Chl combination as first line treatment in MCL patients with other first line treatments in terms of response and safety. **Patients and Methods.** Patients diagnosed with MCL using established criteria in 6 Hematologic Centers in Greece, from June 2003 until August 2011, were included in this study. Patients with the blastic variant of the disease were excluded from the analysis. They were classified in 4 groups according to the treatment protocol administered as follows: - First group (A): patients received the R-Chl combination - Second group (B): patients received the Rituximab-CHOP regimen - Third group (C): patients received the HyperCVAD regimen - Fourth group (D): patients received other therapies. **Results**. 65 patients (51 male) were analysed in the study. Their median age was 69 years (range 32-97)Twenty-three patients (36%) classified in group A,24 patients (37%) in group B,while 10 (15%) and 8 patients(12%) in groups C and D respectively. In group D, 5 patients received the Rituximab-COP combination, 1 received Riruximab as a single agent while 1 received the FCR regimen and 1 an mTOR inhibitor. Three patients(12%) in group A were in high intermediate/high prognostic group according to IPI score whereas 13(54%), 5(50%) and 6(75%) patients in groups B,C and D respectively. Moreover 2 patients were in high prognostic group according to MIPI score while 9(38%), 3(30%) and 4(50%) patients in groups B,C and D respectively. Treatment toxicity is shown in Table 1.

Table 1.

Group	A	B	C	D
		#	(%)	
Neutropenia grade III-IV	–	9 (38)	8 (80)	–
Febrile neutropenia	–	2 (8)	6 (60)	–
Anemia grade 3-4	–	3 (13)	5 (50)	–
Thrombocytopenia grade 3-4	–	–	8 (80)	1 (13)
Toxic Deaths	–	–	1 (10)	–
Hospitalization #patients -Median duration (days)	4-(10)	10-(12)	8-(95)	2-(4)

5-year overall survival was 70% for all patients while 5-year overall survival for the patients received the R-Chl combination and for those received therapies other than the aforementioned combination was 90% and 60% respectively (p:0,21). **Conclusions.** High response rates were achieved by both the aggressive and less aggressive treatment regimens. Patients who received aggressive therapies presented with more unfavorable disease characteristics compared to those who received the R-Chl combination and demonstrated more frequent severe complications attributed to therapy. The R-Chl combination is extremely efficient especially for a fraction of patients who have indolent disease characteristics.

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CLINICAL FEATURES PRIMARY EXTRANODAL DIFFUSE LARGE B-CELL LYMPHOMA (PEDLBCL)

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Background. PEDLBCL arise from tissue other than lymph nodes and even from sites which normally contain no lymphoid tissue. The purpose of our work was revealing of adverse prognostic factors. **Methods.** We evaluated the clinical features and outcomes 42 patients who were treated in Russian Blockhin Cancer Research Center between 2000 and 2010. **Results.** Localisation of the primary lesion was important prognostic factor: 5-years overall survival(OS) was 88% in stomach lymphoma, 80% in bones lymphoma, 60% in testis lymphoma;(p=0,4). 5-years event free survival(EFS) was 75% in stomach lymphoma, 70% in bones lymphoma, 50% in testis lymphoma;(p=0,6). Patients age varied from 19 till 82 years (median was 56 years). Patients more 60 years was 18 (43 %). 5-years OS ≥60 years old was 61 %, and patients <60 - 83%;(p=0,03). 5-years EFS elderly was - 56%, at young age - 79% (p=0,03). I-II stages have been established at 26 cases(61 %), IV stage was established at 16 cases (39 %) PEDLBCL. If at I-II stages 5-yearsOS - 77%, at IV - 63%(p=0,2). 5-years EFS at the localised stages of disease was 69%, and at advanced stage - 56%(p=0,4). At case involving 1 extranodal zone 5-years OS it was equal 80%, more than 1 zones - 68%(p=0,6). 5-years EFS at involved of 1 zone was 73%, at case of the widespread form disease 55%(p=0,5). The general ECOG condition, corresponding 0-1 points, has been noted at the majority of patients - 26 (62%). 5-years OS was 62% at ECOG>2, and 81 % at ECOG 0-1(p=0,2). 5-years EFS in poor performance status at the moment of beginning treatment was53 % and at a satisfactory condition - 69%(p=0,2). LDH>N was in 40 % (17 from 42 cases): 5-years OS at ЛДГ ≤450IU - 81%, at ЛДГ >450IU - 62%(p=0,8). 33 (78%) patients were included into group of the favorable prognosis (low and low/intermediate groups of risk of early progressing), and 9 (22%) the adverse prognosis (high/intermediate and high degrees of risk of early progressing). 3-years OS was 56 % at patients with high and high/intermediate risk and 86 % at patients with low and intermediate/low risk (p=0,3). Distinct negative prognostic value renders primary refractory to chemotherapy: 3-years OS was 93% at achievement complete remission, and 36% at patients with resistant form of disease (p=0,0001). **Conclusions.** Unfavourable prognostic factors are nodal localisation of the primary disease lesion, the age is more 60 years, ECOG score >2, LDH level >450IU,advanced disease at the moment of diagnostics (IV stage, involving more than 1 extranodal zone, «bulky»), high/intermediate and high degrees of risk of early progressing, resistant form of lymphoma to I line therapy.

1733

PROGNOSTIC SIGNIFICANCE OF CD5 IMMUNOEXPRESSION IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH IMMUNOCHEMOTHERAPY

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Background. CD5+ DLBCL is a clinically distinct subgroup of DLBCL that is associated with poor prognosis. **Methods.** In this study, we have analyzed immunoexpression of CD5 in group of 55 DLBCL patients treated with immunochemotherapy. The diagnosis of DLBCL was made according to the Revised European-American Lymphoma Classification. The expression of CD5

has been evaluated semiquantitavely as a percentage of tumor cells. **Results.** Immunoexpression of CD5 has been found in 10(18. 2%) patients. Expression of CD5 ranged from 30 to 100%. Patients with CD5+ DLBCL was more closely associated with many aggressive clinical features or parameters than CD5- DLBCL: Namely, immunoexpression of CD5 was in significant correlation with advanced clinical stage (III, IV) (p=0. 023), performance status (p=0. 036) and presence of extranodal localization (p=0. 047). The overall International Prognostic Index score was thus significantly higher for the patients with CD5+ DLBCL than for those with CD5- DLBCL (p =0. 016). Immunoexpression of CD5 was found to be unfavorable factor for therapy response (p=0. 026). In addition, the patients with CD5 expression had significantly the shorter survival than CD5+ shorter survival (67. 1 vs 30. 2 months) (p=0. 012). **Conclusions.** These results confirmed that CD5+ DLBCL constitute an unfavorable prognostic subgroup of DLBCL in patients treated immunochemotherapy.

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CYCLOPHOSPHAMIDE AND METHOTREXATE ORAL COMBINATION CHEMOTHERAPY REGIMEN FOR NON-HODGKIN LYMPHOMA: LOW DOSE METRONOMIC THERAPY

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Background. Metronomic chemotherapy, which is defined by the frequent, repetitive administration of chemotherapeutic drugs at relatively low doses, and without prolonged drug-free break, is an emerging strategy to fight cancer. The mechanisms of metronomic therapy have been unveiled as tumor anti-angiogenesis and multitargeted therapy. Many patients, especially elderly person, with recurrent lymphoma are unable to tolerate intensive therapies. Metronomic chemotherapy offers a novel, potentially less toxic yet effective treatment strategy. **Aims.** We assessed the response, progression-free survival, overall survival and the toxicity of metronomic chemotherapy in patients with non-Hodgkin lymphoma. **Methods.** A retrospective analysis was performed on 13 non-Hodgkin lymphoma patients (6 male, 7 female) who were treated with cyclophosphamide and methotrexate (CM) regimen for more than one month. They received oral cyclophosphamide (50 mg every day) and oral methotrexate (2. 5 mg 2 times/week) until there was disease progression or unacceptable toxicity. The study was conducted in accordance with the Declaration of Helsinki including all current amendments, and the study protocol was approved by the local ethics committee. **Results.** Among 13 patients (median age 80 years, range 44-88), 6 patients had diffuse large B cell lymphoma (DLBCL), 2 mantle cell lymphoma, 2 angioimmunoblastic T cell lymphoma, 1 MALToma, 1 marginal zone B cell lymphoma (MZBCL) and 1 small lymphocytic lymphoma (SLL). CT regimen was used as 1st line therapy for a DLBCL patient who had refused standard R-CHOP chemotherapy, and used as maintenance therapy for a MZBCL and a SLL patient. Others were relapsed / refractory lymphoma patients. Thirty-one percent of patients had previously received 2 or more treatments. Twelve of 13 patients are evaluable for response. The overall best response rate is 46% (2 complete response and 4 partial response), with 15% achieving stable disease. Median treatment duration was 4. 8 months (range 1. 5 - 25. 7). Twelve patients discontinued the therapy and the reasons were disease progression (7), infection (2), poor performance status (1) and patient's or doctor's decision (2). Median overall and progression-free survivals were 27. 1 and 9. 9 months, respectively. The median response duration was 15 months. Only 4 patients could be managed with further salvage chemotherapy. The CM regimen was generally well tolerated. The most common toxicity was myelosuppression (leukopenia 77%, Grade 3 23%); and two patients with grade 3 infection were reported. There was no treatment related mortality. **Conclusions.** Metronomic therapy with oral CM regimen administered for continuous, prolonged periods represents an active, easily tolerated approach to management of patients with recurrent non-Hodgkin lymphoma, particularly for extremely old patients who have difficulties to apply aggressive salvage chemotherapy or hematopoietic stem cell transplantation.

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HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL SUPPORT FOR PRIMARY CNS LYMPHOMA: A SINGLE INSTITUTE EXPERIENCE IN JAPAN

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Background. The prognosis of primary CNS lymphoma (PCNSL) still remains dismal, with high rates of local relapse and consequent death. Although many groups have reported that high dose chemotherapy with autologous stem cell transplantation (HDC/ASCT) improve survival in PCNSL patients, the treatment is not widely used in Japan. We report the results of HDC/ASCT for PCNSL in our hospital. **Methods.** Immunocompetent patients with PCNSL were included. Treatment consisted of two to four courses of HD-MTX/CHOP +/- rituximab and two to three courses of HDMTX/IFO +/- rituximab, then stem cell harvest, followed by HDC/ASCT. Progression free survival (PFS) was measured from start of chemotherapy to first documentation of progression, last follow up or death. **Results.** Between June, 2009 and January, 2012, nine patients underwent ASCT for PCNSL. Median age at diagnosis was 64 years old (range 56-74). Median time from diagnosis to transplant was 6. 4 months (range 4. 8 to 7. 2). Disease status at transplant: First CR 8 patients, PR one patient. The median $CD34^+$ cell number collected was 1. 97 x 10^6/kg body weight (range, 1. 4 to 3. 6). The median numbers of leukaphereses was four (range two to six). Median follow-up period after ASCT was 13. 1 months (range 1. 1 through 33. 7). The first five patients received Busulfan/Thio-tepa conditioning, whereas the others received Busulfan/Melphalan conditioning, due to unavailability of Thio-tepa after 2010 in Japan. Two patients have relapsed at 5. 7 and 7. 7 months after ASCT. Both two patients are alive after additional therapy. Two patients died by respiratory distress and an accident. Median overall survival and progression free survival from transplant have not been reached. Two year overall and event free survival are 72. 9% and 68. 6%, respectively. **Conclusions.** Although there is limitation related to unavailability of the drugs in Japan (e.g., thio-tepa, BCNU), our result suggests that HDC/ASCT for PCNSL demonstrates improved overall survival when compared to historical controls. Further prospective study is warranted.

1736

VERY GOOD OUTCOME OF PRIMARY GASTRIC DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) AFTER CONSERVATIVE TREATMENT INCLUDING CHEMOTHERAPY WITH OR WITHOUT RITUXIMAB

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Background. Gastric DLBCL has been treated with various modalities including surgery, chemotherapy, and radiotherapy alone or in combination. Historically, surgical resection of tumor was believed to be the mainstay of treatment with or without additional therapy Surgical resection surely provides definitive diagnosis and reduces the tumor bulk immediately. This approach, however, was questioned and several studies showed the benefit of conservative treatment with chemotherapy with or without radiotherapy. **Aims.** To retrospectively evaluate the efficacy of conservative treatment with CHOP chemotherapy with or without Rituximab in primary gastric DLBCL. **Methods.** Between 8/2001 and 9/2011, 19 nongastrectomized patients (pts) with gastric DLBCL have been admitted in our institution. Diagnosis was made by endoscopic histologic specimens without gastric surgery in all cases. Disease evaluation was performed with gastroscopy, whole-body computed tomography and bone marrow biopsy at diagnosis and at the end of treatment. Until 12/2004, treatment consisted of CHOP/CHOP-like chemotherapy (5 pts) and, subsequently, of CHOP/CHOP-like chemotherapy in combination with Rituximab (14 pts). All pts received supportive oral treatment with proton pump inhibitors and antiacids. Corticosteroids were administered intravenously or intramuscularly. **Results.** Eleven patients were male and 8 female, with a median age of 62 years (46-81). According to Lugano staging system,9 ptswere in stage I,6 ptswere in stage II-1,2 ptsin stage II-2 and1 ptin stage IIE and IV respectively. B-symptoms were present in8 ptsand aaIPI 2-3 in2 pts. At diagnosis, endoscopy showed neoplastic ulcers in14 ptsand HP positive specimens in7 pts. Today 18/19 pts completed planned treatment. Pts received a median number of 6 courses of chemotherapy with or without Rituximab. After CHOP/CHOP-like chemotherapy alone, 3/5 pts (60%) achieved complete remission (CR). The 2 partial responder pts achieved CR after radiotherapy and partial gastrectomy, respectively. All14 ptswho received R-CHOP combination achieved CR (100%). After a median follow up of 58 months (6-126) OS and RFS are 100%. Considering as treatment failure the need of adjunctive radiotherapy or surgery after chemotherapy, only 2 failures occurred in the group of pts treated without Rituximab. Finally, all pts were treated as outpatients and we didn't observe gastrointestinal bleeding or perforation during treatment. **Conclusions.** In the recent years, conservative approaches such as radiation therapy and / or chemotherapy are generally favoured as opposed to surgical resection in the management of primary DLBCL, because of morbidities and poor quality of life associated with gastrectomy. The role of radiotherapy had been established in the management of limited-stage DLBCL in the preprituximab era. However, radiotherapy may not be preferred today as much considering the long-term toxicity of radiation exposure. Rituximab has an established role in the management of DLBCL, but information on its role in primary gastric DLBCL is limited. However, in our experience R-CHOP combination was a safe and highly effective therapy for gastric DLBCL and represent, now, gold standard treatment for pts affected by Gastric lymphoma in our institution.

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T(14;19)(Q32;Q13) IS POOR PROGNOSTIC FACTOR IN SPLENIC MARGINAL ZONE LYMPHOMA

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Background. Splenic marginal zone lymphoma (SMZL) is a well recognized B-cell neoplasm which is characterized by splenomegaly, bone marrow involvement, immunologically by typical phenotype of marginal zone cells. The most frequent cytogenetic findings are involvement of chromosomes 1, 3, 7 and 8. The t(14;19)(q32;q13) is a rare cytogenetic abnormality has been reported in B-CLL. **Aims.** To describe the clinical, immunomorphological findings in t(14;19)(q32;q13)-positive SMZL. **Methods and Results.** In Hematological Research Centre, Moscowbetween January 2001 and May 2011 were identified three cases SMZL with t(14;19)(q32;q13). All patients were males with age 51, 58, 67 y. o. Lymphoma presented with B-symptoms, high level of LDH, splenomegaly and enlarged splenic hilar lymphnodes. The hemoglobin was 92 g/l, 110 g/l, 122 g/l. All patients had normal count of leukocytes with an absolute lymphocytosis (lymphocytes count 72 x 10^9 g/l, 79 x 10^9 g/l, 83 x 10^9/l) and thrombocytopenia. Morphological examination of peripheral blood and bone marrow lymphocytes showed that all lymphocytes are atypical with wide cytoplasm and nuclear indentation. In all cases there was nodular type of bone marrow involvement. Immunophenotypic analysis has shown the expression of mature B-cells antigens (CD19, CD20, CD22, FMC7, sIg) and absence of CD10, CD23, CD5, CD43, CyclinD1. Two patients were treated with CHOP-21 regimen without any response. They progressed with spleen enlargement and decreased of thrombocytes counts. All 3 patients undergo splenectomy. Weight of spleen was 1800 g, 2083 g, 2850 g. Histological examinations of spleen specimens show massive nodular pattern (involvement of the white and red pulp) associated with diffuse invasion of the sinuses. In all cases there was high mitotic activity. All patients demonstrated progression after splenectomy during 3-6 months that was characterized by increase of leukocytes count (range 45, 4 - 101, 8 x 10^9 /l), high level of LDH, appearance of peripheral and visceral lymph nodes. Considering the increase leucocytes, presence of lymphadenopathy in all cases CHOP-21, R-FMC regimen were used. All patients died of disease progression and infectious complications. Time of observation was 21, 30, 34 months. **Conclusions.** The t(14;19)(q32;q13)-positive SMZL is distinct variant which is characterized by rapid progression after splenectomy, poor responses to chemotherapy and short survival. So t(14;19)(q32;q13) may be regarded as a poor prognostic factor.

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IMMUNOHISTOCHEMISTRY IN STAGING BONE MARROW BIOPSY SPECIMENS: A USEFUL ADJUNCT FOR MORPHOLOGICAL DIAGNOSIS IN NON HODGKIN'S LYMPHOMA

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Background. Morphological examination of bone marrow trephine biopsy represents a standard method for non-Hodgkin's lymphomas (NHL) staging. Immunohistochemistry staining of bone marrow trephine specimens by using a panel of antibodies has been used in haematological malignancies due to its high applicability and sensitivity at diagnosis. However, the routine use of immunohistochemistry in the clinical settings when there is no obvious morphological (light microscopic) evidence of lymphoma in the bone marrow trephine, is not yet well established. **Aims.** To assess the value of immunohistochemistry (by using a standard basic panel of anti-CD20 and anti-CD3 staining) in detecting involvement by NHL in routinely processed bone marrow trephine specimens, which show no obvious morphological involvement with lymphoma. **Methods.** This study involved 38 randomly selected paraffin wax embedded, formalin fixed bone marrow trephine specimens from three teaching hospitals between February 2011 and February 2012. Thirty patients (79%) had B-cell NHL: diffuse large B cell lymphoma (DLBCL) 45% (n=17), follicular lymphoma (FL) 18% (n=7), marginal zone lymphoma (MZL) 10% (n=4), mantle cell lymphoma (ML) 8% (n=3), Burkitt's lymphoma (BL) 5% (n=2). Eight cases (21%) had T/NK lymphomas. There was no obvious morphological evidence of bone marrow infiltration as all samples were reviewed by two examiners. All specimens were stained with the anti-CD20 and anti-CD3 antibodies. **Results.** Concordant results were found in 35 samples (92%), as both investigations were reported negative. 3 of the 38 cases (8%) which showed no morphological evidence of involvement by NHL on routine stains, were positive on immunohistochemistry. Considering histology, discrepant results were noted more frequently in T/NK lymphomas (25%; 2 of 8 cases) comparing to B-cell NHLs (3%; 1 case of DLBCL). In all three cases the lymphoid infiltrates had diffuse pattern. **Summary and Conclusions.** Our results indicate that immunohistochemistry can detect a subgroup of NHL patients with bone marrow involvement beyond discriminatory level of conventional stains (Haematoxylin & Eosin and Giemsa), thereby contributing to accuracy of staging. In our view, rational application of immunohistochemistry is a cost-effective & valuable method in routine investigation of staging bone marrow trephine biopsies.

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ULTRASOUND-GUIDED TRANSTHORACIC BIOPSY WITH FULL-CORE CUTTING NEEDLE TRUCUT IS EASY, EFFECTIVE AND SAFE FOR THE HISTOLOGICAL DIAGNOSIS OF MEDIASTINAL LYMPHOMA

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Background. The development of a mediastinal mass is often associated with a clinical emergency. Primary mediastinal B-cell lymphoma (PMBCL) is usually characterized by symptomatic superior vena cava syndrome and pleural or pericardial effusions with cardiac tamponade at diagnosis. Fine-needle aspiration is a well-established technique to obtain cytological specimens, but it is not useful to a correct lymphoma characterization. Nowadays, mediastinoscopy or invasive thoracic surgery still represent the standard procedures to provide diagnosis in this subset of patients. **Aims.** The aim of the study was to determine the contribution of ultrasound (US)-guided transthoracic biopsy with full-core cutting needle trucut in the diagnosis of PMBCL. **Methods.** By the end of 2010, patients afferred to our Institution for suspected diagnosis of lymphoproliferative disease with bulky mediastinal mass were evaluated by a multidisciplinary team. The team consisted of an hematologist, a thoracic surgeon and a radiologist expert in ultrasonographic vascular assessment. A computed-tomography assessment of the thorax was performed for staging. In presence of a lymph nodal mass of the anterior mediastinum, patients underwent US-guided transthoracic biopsy with full-core cutting needle trucut. Biopsy was performed with BioPince Full-Core biopsy instrument, 16 Gauge. BioPince technology was based on "tri-axial"cut and capture cannula system, designed to harvest diagnostic quality specimens while reducing the risk of crush artefact and tissue fragmentation. **Results.** From December 2010 to October 2011, six patients with clinical suspect of primary mediastinal B-cell lymphoma with anterior mediastinum mass underwent US-guided transthoracic biopsy with full-core cutting needle trucut. All patients were women, median age was 32 (19-50) years. Clinical emergency was determined by: superior vena cava syndrome in four, three of them with pericardial effusion with cardiac tamponade, vocal cord paralysis in one and endovascular dissemination with compression of innominate vein. US-guided transthoracic biopsy with full-core cutting needle trucut was performed at a median of two days after first clinical evaluation. All patients underwent chest X-ray after biopsy; no complications were observed. No patients required hospitalization for the procedure or prolongation of the hospitalization. All biopsy were successful, with proper harvested cylindrical full core specimens. At least two or three samples were collected in each biopsy for histological diagnosis; median length of harvested specimens was 1. 2 cm, range 0. 3 cm to 2. 3 cm. Histological characterization, with immunohistochemical analysis and Ki67 evaluation, was available in all cases. This technique allowed to start first infusion infusion of chemotherapy at a median of 8 (3-14) days after first clinical evaluation or 5 (2-11) days after biopsy. **Conclusions.** US-guided transthoracic biopsy with full-core cutting needle trucut was a promising technique in diagnosis of primary mediastinal B-cell lymphoma or other mediastinal mass. This not invasive approach allowed good quality of histological specimens, with shortening of the time of diagnosis and rapid starting of chemotherapy treatment.

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PRIMARY BONE LYMPHOMA - THE REPORT OF 19 CASES

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Background. Primary bone lymphoma is a rare entity (5%) of extranodal non-Hodgkin lymphoma. Predominant manifestations at presentation are pain,

pathological fracture or appearance of a tissular mass. Approximately (50%) of presented cases were in stage IV of the disease. Histopathologically, primary bone lymphoma represents a diffuse B large cell lymphoma. Aim of study: to analise a group of 19 patients with primary bone lymphoma. **Methods.** We have reviewed the clinical manifestations, outcome and treatment of 19 patients diagnosed with primary bone lymphoma, between 1997 and 2011, in the Clinic of Hematology from Craiova . Five patients were females and 14 patients were males, aged 67 between 79. The inaugural symptom was pain (8 patients), a soft a tissue mass (4 patients) and pathological fracture (7 patients). Disease localisation at diagnosis was in the tibia (9 patients), the vertebra (6 patients), the mandibula (3 patients) and the femur (1 patient). 50 % of patients were in stage I of disease and 50% in stage IV. All patients were treated with chemotherapy. CHOP (7), BLEO-CHOP (12), followed by radiotheraphy to the primary site. Autologous blood stem cell transplantation was performed in two cases. **Results.** Nine patients out of 19 achieved complete response and relapse was observed in 4 patients. Nine patients died due to the progresson of the disease. Up to now, 10 patients are alive with no evidence of disease (52%). **Conclusions.** Optimal regimens for the treatment of primary bone lymphoma have not been firmly established. Anyway, our observations and literature data indicate that the response rate and the disease free survival is higher with the combination of radiotherapy and chemotherapy.

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TREATMENT Results OF PATIENTS WITH PRIMARY NON-HODGKIN'S LYMPHOMA OF THE TESTIS- A RETROSPECTIVE STUDY FROM A SINGLE HEMATOLOGICAL CENTRE

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Background. Primary testicular lymphoma is a rare subgroup among extranodal non-Hodgkin's lymphomas(nHl) with poor prognosis because of its aggressive clinical behaviour due to high grade histological features developed in mainly elder population. Orchiectomy followed by combination immunochemotherapy are traditional treatment methods with a rather inferior outcome. **Aims.** In a retrospective survey we discuss the clinical presentation,patholgical feature and treatment results of patients (pts) with primary testicular lymphoma (PTL) diagnosed and treated in our hematological centre between 2000-2011. **Patients and Methods.** During this period from 313 pts treated with aggressive nHl in eight pts (age 23 to 86, median of 60 years) with testicular swelling underwent to semicastration primary testicular lymphoma (in seven pts with diffuse,large B-cell origin and in one with Burkitt-like lymphoma)was diagnosed (8/313, 2. 5%). According to the Ann Arbor staging system limited stage I-IIE in seven, advanced stage in one patient were observed. All pts except one (only radiotherapy)were getting rituximab added to CHOP regimen(R-CHOP,6 or 8 cycles in every 21 or 28 days). Central nervous system (CNS)prophylaxis was used in only case and no contralateral testis preventive irradiation was used. **Results.** With a median follow-up of 38 months,after semicastration used R-CHOP treatment resulted in seven pts complete remission, however, two pts died(one due to progression, one other in remission from pulmonary solid tumour). Disease-free(1-140,median of 32 months)and overall survival rates (5-144,median of 38 months) were achieved. 3-year DFS and OS both revealed to be 50%, respectively. **Conclusions.** Our relatively favourable treatment results could mainly be explained with the excess of pts with early-stages in which after early surgical remove of testicular lymphoma combined immunochemotherapy seemed to be sufficient to prevent localized and also distant relapses,in spite of no regular prophylaxis of the CNS,relapse was also not occured.

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PRIMARY CUTANEOUS DIFFUSE B LARGE CELL NON-HODGKIN LYMPHOMA IN FIRST-DEGREE BLOOD RELATIVES

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Aims. The occurrence of non-Hodgkin's lymphoma (NHL) in several members of one family is uncommon and the real risk of lymphoma aggregation and mechanisms are uncertain. We present two brothers, whose mother died of gastric NHL, in whom in a period of 4 years cutaneous B diffuse large cell non-Hodgkin lymphoma appeared on the skin of the head and resolved on immunovhemotherapy with R-CHOP (rituximab and adriblastine, cyclophospahmiede, oncovine and prednisone). **Case Reports.** Case 1. A 44-year-old man, anesthesiologist, presented with a 16-year history of occipital and parietal alopecia and scalp itching. During the last year of the disease on the scalp appeared small nodules and crustae. The treatment with cortiocsteroids had no effect on the lesions. In November 2006, standard laboratory data indicated that the patient had a leukocytosis of 29 x 109/l (seg 69%, lymphocytes 20%, monocytes 11%), a fibrinogen of 7. 76 g/l and a mildly elevated LDH at 680 U/l (normal values 313-618 U/l). Bone marrow aspirate and biopsy, abdominal, chest and pelvis CT scan were negative for systemic lymphoma. CT scan of the head was normal except for the scalp soft-tissue abnormality. Histology from a scalp nodular biopsy showed dermal lymphoid infiltrates with numerous centroblasts, immunoblasts and centrocytes. Imunohistochemistry of the neoplastic cells showed that they were CD20+,CD79alfa+, bcl-2-, CD30-, CD3-, CD5-, CD10-, bcl-10-, MUM-, bcl-6+, CD138-. Ki-67, was positive in up to 60-80% cells. This finding corresponds to a transformation of the low-grade PCFCL to a high-grade diffuse large B-cell lymphoma. The patient was treated with R-CHOP protocol with excellent response and he is in remission ever since. Case 2. A 44-year male, truck driver presented with itching, alopecia and small cutaneous livid nodules on the scalp, the largest in diameter 2x2. He was treated with protocol CHOP for B NHL of the same localization 26 years earlier in his 18 year of life. He was in remission since than. Otherwise physical finding was normal. Chest and abdominal CT scan was normal. Bone marrow biopsy showed normal haematopoiesis. Laboratory data including CBC were in the normal range. Histology of the scalp nodules showed dermal and hypodermal infiltration with tumor tissue composed of large cells, with scarce cytoplasm and large oval, or irregular vesicular nucleuses, some were multinucleated. Immunohistochemistry of the neoplastic cells was as follows: Tdt-, PAX-5+, CD20+, CD3-, CD5-/+, CD10+/-, bcl-2-/+, MUM-1-, CD30-, cyclin D1-, Ki-67+ in 80% of cells. The finding corresponds to high grade diffuse B large cell lymphoma. The patient is under R-CHOP treatment with excellent response. **Conclusions.** Primary cutaneous non-Hodgkin's lymphoma (PCNHL) is a heterogeneous group of lymphoproliferative disorders characterized by their indolent course, virtually exclusive skin involvement and the absence of systemic disease. The presented cases are consistent with previous findings of familial aggregation of NHL. In this case environmental risk factors are unknown but as they were living together it is possible that environmental factors in addition to genetic might have played some part in the development of NHL in this family.

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THE EFFICACY OF INTERFERON-α IN NON CUTANEOUS PERIPHERAL T CELL LYMPHOMAS

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Background. Peripheral T- cell lymphomas (PTCL) are a heterogeneous group of uncommon malignancies that represent approximately 12% of all lymphomas. Prognosis is poorer compared to B cell counterpart and despite various intensive regiments relapses are very common. Interpheron-α (INF-α) has long been known as an active agent in a variety of diverse malignancies. **Aims.** Based on the knowledge of successful management of cutaneous T cell lymphomas (T-NHL) with INF-α, we present our experience on administrating INFα in patients with non cutaneous PTCL. Patients and **Methods.** The retrospective study included 15 patients with non cutaneous PTCL who were treated with INF-α in our departments at a point of their disease course between February 1992 and July 2007. Patients received INF-α at a dose of 3-5MUI per day or 3-5MUI three to four times per week and the minority of them pegylated INF-α due to adverse reactions of classical INF-α. We isolate and present the group of 5/15 patients who received INF-α after failing standard therapy approach or as first line treatment due to personal reasons and achieved long term complete remissions (CR). **Results.** 2 male and 3 female patients with a median age of 30 years (range 18-50), two with T-NHL NOS, one with anaplastic large cell lymphoma (ALCL) ALK unknown, one with ALCL ALK+ and one with ALCL ALK- were studied. All 5 patients at diagnosis were either clinical stage I or II, with a good performans status, absence of B symptoms, no bulky disease and LDH within normal range. 3/5 patients had extranodal location of the disease at diagnosis (2 tongue, 1 lip). All but one received INF-α beyond first line treatment with combined chemotherapy (two second line treatment and two third line treatment after rescue and autologous stem cell transplantation). 3/4 patients were treated with CHOP regiment and 1/4 with ALL regiment as first line treatment. 4/4 patients relapsed after a median time of 7 months (range 2 to 30). One patient received INF-α as first line treatment due to 12th week of pregnancy at diagnosis. 5/5 patients are in CR, with a median follow up of 53 months (range 12 to 83) (Table 1). No major adverse reactions were noticed and all 5 patients

continued their treatment with no need of discontinuation. **Conclusions.** INF-α may have a beneficial role in a subset of patients with non-cutaneous PTCL who are refractory to standard therapy. These findings need to be further studied.

Table 1.

Pt	A/G	Dgn	Clinical stage	IPI	Reg. before INF Initiation	TTRel after 1st line Treatment	INF Cycles	Current Status
1	30/M	ALCL ALKu	IIA	low	*CHOP	8months	48	Alive CR2
2	18/M	ALCL ALK+	IIA	low	*ALL *ESHAP/ ASCT *HyperCVAD	2 months	24	Alive CR3
3	32/F	NOS	IEA	low	-	-	53	Alive CR1
4	50/F	ALCL ALK-	IEA	low	* CHOP	30 months	83	Alive CR2
5	22/F	NOS	IEA	low	*CHOP *ESHAP/ ASCT	4 months	64	Alive CR3

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MATURE T CELL LYMPHOMA: EPIDEMIOLOGIC FEATURES AND THERAPEUTIC RESPONSE

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Background. T Cell Lymphoma accounts for only about 10% of all cases of Non-Hodgkin Lymphoma. **Methods and Aims.** We conducted a retrospective study including patients diagnosed with mature T cell lymphoma between 2001 and 2010 to determine the epidemiological features, the prognostic factors and to focus on therapeutic outcomes. **Results.** 43 patients were diagnosed with a median age of 47 (10-84). A male preponderance was noted with a sex ratio (M/F) of 3. 2. Systemic symptoms and PS (>=2) were present respectively in 41 % and 68 % of patients. The most common histological subtypes were: peripheral T cell (NOS) (35% of cases), Anaplastic T Cell lymphoma (ALCL) (25%), Angioimmunoblastic lymphoma (AIL) (25%) and T/NK nasal type in only 4 patients (9%). Advanced stages (III-IV) were observed in three quarters of patients. Organ involvement was found in 24 patients (55.8%). Extranodal spread of the disease with BM being the most frequent site (37.5%) followed by lungs (25%), liver (12.5%), skin (8. 3%), pleura (8.3%) and adrenal glands(8. 3%). Bulky disease was present in 8 patients (18.6%). The laboratory investigation showed elevated serum LDH (> 1normal) in 27 cases (62%). IPI scoring was available in all patients, accordingly 29 patients (67. 4%) had an IPI≥ 2 and 14 patients (32. 5%) had an IPI (0-1). 3 patients died before any treatment. The patients older than 65 years were treated by a mini CEOP regimen with a median number of courses of 6 (1-8), those under 65 years old received standard CHOP and CHOP-like regimen (CHOEP, ACVBP) with a median number of cycles of 4 (1-8). 2 children aged of 10 and 11 years received pediatric regimens, respectively LMB01 C3 and EORTC protocols. Only 32 patients were assessable for treatment response: 13 patients achieved CR (32.5%), 11 patients had PR (27. 5%), 3 patients had stable disease (SD) (7.5%) while 5 patients progressed. 5 patients died before assessment of treatment response with mortality rate of 12. 5 %. Response therapy was not recorded in 3 patients. Relapse rate was 25%. All relapses occurred within the first 12 months. The autologous SCT was performed in 7 patients, among them 5 were in first response. The median overall survival (OS) and event-free survival (EFS) were respectively 15. 25 and 17. 75 months. In univaried analysis, none of the following factors (LDH, stage, IPI) significantly affected OS and EFS. **Conclusions.** The epidemiological characteristics were similar to published data; however, our therapeutic results were poor compared to literature. In our series, no factor had been identified as significantly associated with poor OS and EFS due to the sample size.

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RA-36, NOVEL THROMBIN BINDING DNA APTAMER EXHIBITS HIGH INHIBITORY POTENTIAL AND SPECIFICITY FOR BLOOD COAGULATION

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Background. G-quadruplex-based DNA aptamers for human thrombin represent promising pharmaceuticals having high anticoagulant activity, rapid clearance from the bloodstream, and availability of DNA antidote. The successful examples are well described minimal G-quadruplex 15-TBA (Bock et al., Nature, 1992, v. 355, 564-566), elongated with hinge and duplex regions 31-TBA (Ikebukuro et al., Nucl. Acids Res., 2005, v. 33, e108), and developed recently modular aptamer RA-36 (PCT 2010/000750, December 13, 2010). RA-36 was shown to possess high inhibitory efficiency and low toxicity *in vivo*. **Aims.** High specificity of aptamers minimizes side-effects but at the same time leads to difficulties when choosing animal model for preclinical trials. In this work target- and species-specificity of DNA aptamers were investigated thoroughly. **Methods.** Enzymatic turbidimetry-based assay and fibrinogen and plasma clotting assays were used. **Results.** The experiments with human, bovine, porcine, rabbit, rat, and mouse thrombins have revealed the species-specificity of aptamers. In particular, mouse and rabbit thrombins gave heightened IC50 values in enzymatic and clotting assays. 15-TBA was shown to inhibit human, bovine, porcine, and rat thrombins with the same inhibition constants and types; whereas 31-TBA and RA-36 inhibited these thrombins with variable efficiency and inhibition type indicating that the G-quadruplex elongation does implement the subtle regulation of thrombin-fibrinogen interaction. A set of tests on blood clotting cascade was performed to investigate target-specificity of the aptamers. RA-36 aptamer was shown to inhibit plasma and blood clotting in all tests implied thrombin addition or generation and not to inhibit all other tests, including amidolytic activity of thrombin. The only exception represents ecarin and reptilase tests, which implies, correspondingly, meizothrombin and reptilase as fibrinogen clotting reagents. Thus, some cross-reactivity was observed for proteins with fibrinogen binding site. **Summary.** Given all together, the results indicate high target- and species-specificity of RA-36 characterizing it as new promising anticoagulant with minor side-effects. This work was supported by the Ministry of Education and Science, the State contract №- 16. 512. 11. 2009.

1746

OXIDATIVE STRESS MEDIATED APOPTOSIS BY DISULFIRAM/COPPER COMPLEX IN CD34+CD38-KG1A CELLS IS REGULATED BY C-JUN-AMINO-TERMINAL KINASE AND NF-?B PATHWAY

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Background. Acute myeloid leukemia (AML) is a hierarchical disease initiating by a rare population of cells known as leukemia stem cells (LSCs), which play the central role in the relapse and refractory of AML and represent a critical target for therapeutic intervention. CD34+CD38-KG1a cells, a subpopulation of the KG1a leukemia cell lines, have been demonstrated previously to meet the properties of stem cells and could be used as the cell model for LSCs research. Previous studies indicated that Disulfiram (DS), an anti-alcoholism drug, have a significant potential in the treatment of human cancers and this antineoplastic activity was enhanced in the present of copper (Cu). We presumed that DS might have the potential of targeting LSCs in a Cu-dependent manner. **Aims.** To determine the cytotoxicity of DS and DS/Cu complex in LSCs model CD34+CD38-KG1a cells and investigate the underlying molecular mechanisms related with oxidative stress, c-Jun-amino-terminal kinase(JNK) and NF-κB pathways. . **Methods.** CD34+CD38-KG1a cells were sorted from KG1a cell lines by fluorence-activated cell sorting (FACS) analysis. The cytotoxity of DS or DS/Cu complex on CD34+CD38-KG1a cells was detected using MTT assay. Annexin-V/PI and DCFH-DA were employed for apoptosis and intracellular ROS level analysis by flow cytometric analysis. Western blot was adopted for protein expression of NF-E2-related factor 2 (Nrf2), p65, JNK and p-JNK. **Results.** CD34+CD38- cells occupy about (50. 135±4. 53) % of the KG1a cell lines, after FACS sorting, more than 95% of the cells were labeled for CD34+CD38-. DS was highly toxic to CD34+CD38-KG1a cells with IC_{50}48h=6. 269 ±1. 466μM and IC_{50}72h=2. 61 ±0. 931μM, respectively. The cytotoxicity was enhanced by combining use of low concentration of CuCl2 (1μM) with IC_{50}48h=1. 028±1. 017μM and IC_{50}72h =0. 83±0. 937μM. DS induced CD34+CD38-KG1a cells into apoptosis, but the apoptotic population

was massively increased by exposed to DS/Cu complex. Nrf2 is an important regulator to deal with oxidative stress. Western blot showed DS/Cu complex but not DS alone inhibited Nrf2 nuclear translocation. ROS levels are predominantly regulated by the transcription factor Nrf2, consistently. DS/Cu induced intracellular ROS generation. The p-JNK protein expression was up-regulated in CD34+CD38-KG1a cells treated with DS/Cu while p65 protein was down-regulated. In addition, the antioxidant N-acetyl-L-cysteine (NAC) attenuated DS/Cu complex-induced apoptosis, restored Nrf2 nuclear translocation and reversed p-JNK expression. **Conclusions.** DS/Cu complex potentiated cytotoxicity to CD34+CD38-KG1a cells through apoptosis induction. The death-signaling pathway revealed that Nrf2 inhibition and ROS generation, followed by activation of JNK as well as suppression of NF-κB pathways were involved.

1747

CLINICAL FEATURES AND OUTCOME OF 16 PATIENTS WITH PNH TREATED WITH ECULIZUMAB IN SAO PAULO - BRAZIL

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Introduction. Paroxysmal nocturnal hemoglobinuria (PNH) is a very rare clonal hematopoietic stem cell disease characterized by intravascular hemolysis, constitutional symptoms, bone marrow failure and thrombosis. Intravascular hemolysis occurs because erythrocytes become more susceptible to the terminal complement pathway. Eculizumab, a monoclonal antibody against complement C5 fraction, is the first effective drug to treat hemolysis and its consequences in patients with PNH. **Aims.** Our goal was to analyze clinical and laboratory features and flow cytometry studies from 16 patients receiving eculizumab in two centers inSao Paulo-Brazil. **Methods.** The Informed Consent was obtained by personal interview with all patients that participated in this study. Sixteen consecutive patients with PNH were prospective analyzed. Patients were followed clinically and by routine laboratory tests in order to evaluate response to treatment. Besides, PNH clone size in erythrocytes, granulocytes and monocytes were assessed by multiparametric flow cytometry before and after treatment was started. **Results.** Median age was 35 years (range: 22-81), 10 (63%)were female and most patients (11 cases - 69%) had classical PNH. Two patients had aplastic anemia and 3 had myelodysplastic syndrome in association with PNH. All patients had frank chronic hemolysis. All patients had large granulocyte and monocyte clones (at least 50%). Median clone size was 30% in red blood cells (range: 5-63%), 78% in neutrophils (range: 55-99,5%) and 88% in monocytes (range: 69-99,9%). Eight patients (50%) had history of venous thromboembolism and 4 of these (50%) were still receiving anticoagulation at the eculizumab was started. Three patients were receiving anticoagulation for primary prophylaxis. Median time between onset of symptoms and diagnosis was 18 months (range: 0-85), and between diagnosis and eculizumab treatment start was 40 months (range: 2-267). Median follow-up time was 57 months (range: 8-286); and median time of eculizumab treatment was 12 months (range: 1-41). There was a significant reduction in the LDH levels: median LDH level was 1841 U/L (range: 797-4319) pre-treatment and 501U/L (range: 200-789, $p < 0.0001$) at week 5. There was also significant reduction in transfusion requirement, most patients becoming transfusion independent. There was also a significant improvement in quality of life assessed by FACIT (functional assessment of chronic illness therapy) questionnaire. After eculizumab was started, there were no episodes of venous or arterial thromboembolism. There was no significant change in clone size after eculizumab treatment. **Summary and Conclusions.** in this series of Brazilian patients, treatment with eculizumab was highly effective in controlling chronic hemolysis, reducing transfusion requirement and improving quality of life.

1748

ENALAPRIL MALEATE HAS CYTOTOXIC EFFECT IN HL 60 LEUKEMIC CELLS BY ALTERING STAT SIGNALING PATHWAY

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Background. Many chemotherapeutic agents have been used in the treatment of acute leukemia, but not yet provided a definitive treatment. Angiotensin converting enzyme inhibitors have been evaluated in many medical conditions other than hypertension including therapy of malignancies due to the potential role of ACE in homeostasis of certain type of tissues such as bone marrow. There are recently some reports indicating the changes of angiotensin-renin system in pathological conditions in bone marrow. **Aims.** In this study, we aimed to evaluate the cytotoxic and apoptotic effects of enalapril on human HL60 acute leukemia cells and to clarify the roles of signal transducers and activator of transcription proteins (STATs) on enalapril induced cell-death. **Methods.** In this study acute leukemia cell line HL60 was treated with enalapril maleate in dose and time dependent manner. Cell viability was assessed at 0, 24th,48th,72 nd and 96 th hour with trypan blue dye-exclusion method and its cytotoxcicity was checked by XTT method. Apoptotic analyses were performed by AnnexinV-EGFP staining method and fluorescence microscopy. Expression levels of STAT3, -5A and -5B genes were analysed in enalapril-treated HL60 cells by qRT-PCR. **Results.** According to the results obtained in our experiments, enalapril was found to induce cytotoxicity in HL 60 cells in dose and time dependent manner. Its IC50 dose was calculated as 7 microM. In HL60 leukemia cells treated with doses of 7 micromolar enalapril, time-dependent expression of STAT3 was investigated with PCR method and it was showed the partially reduced STAT3 expression in enalapril-treated cells when its compared with control group. This reduction was not statistically significant ($p>0.05$). STAT 5A expression was significantly decreased in enalapril treated cells and this difference was statistically significant ($p<0.05$). STAT 5B expression was not changed significantly in enalapril-treated cells when its compared with control group ($p>0.05$). When we assessed the apopitosis with Annexin V method in enalapril treated HL-60 cells, it was detected that there was a slight increase in the number of apoptotic cells within first 48 hours. **Summary and Conclusions.** Taken together all these data showed for the first time that enalapril has significant anti-cancer potential for the treatment of acute leukemia. It is needed to have further studies to clarify the interaction of signalling pathways as an underlying mechanism of enalapril-induced cytotoxicity in HL60 leukemic cells.

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PRACTICAL EXPERIENCE WITH A NEW APPLICATION MODE OF RITUXIMAB: A RETROSPECTIVE SURVEY ON THE ADMINISTRATION OF SUBCUTANEOUS RITUXIMAB AMONG STUDY NURSES INVOLVED IN THE CLINICAL DEVELOPMENT PROGRAM

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Background. Subcutaneous (SC) administration of rituximab has been made possible by co-formulation with recombinant human hyaluronidase (rHuPH20). Compared with standard intravenous (IV) infusion over several hours, rituximab SC may be injected in approximately 6 minutes, typically administered by nurses. This could potentially benefit both patients and healthcare providers. The clinical development program of rituximab SC is based on three randomized clinical trials, which for NHL used a fixed dose of 1400 mg rituximab in an injectable volume of 11.7 ml. Subcutaneous injection of this volume and quantity of monoclonal antibody is unprecedented, and so all aspects of clinical utility and practicality of the rituximab SC injection need to be assessed. Study protocols advise to inject rituximab SC manually into the abdomen using a 27-gauge needle at an injection rate of approximately 2 ml/min. **Objectives.** The aim of the survey was to gain feedback from nurses who are actively participating in rituximab SC clinical trials on different aspects of the rituximab SC administration protocol. **Methods.** A questionnaire in English (later translated into Italian, Spanish, French, and German) was developed by the sponsor for the assessment of nurses' overall impression of using rituximab SC, their experience with the process of rituximab SC administration, aspects of patient care in the context of rituximab SC administration, and potential areas for improvement of the injection. The questionnaire comprised 26 items, with most answers based on a 4-point Likert scale and options for free text answers. Study nurses at investigational sites, who had administered rituximab SC injections to at least one patient, were invited to participate in this internet-based, anonymous, and voluntary survey. The survey was conducted between November 3 and December 19, 2011. **Results.** The survey was sent to 71 sites; 65 questionnaires were completed in full and included in this analysis. The nurses treated a total of 166 patients, with most nurses (63%) having administered 1-5 injections. The majority (72%) reported that SC administration was 'easy' or 'very easy'; however, 55% felt that SC administration of rituximab required a significant amount of physical effort. Approximately, half of those surveyed felt that rituximab SC could be administered at a faster rate without additional stress/pain to the patient, or that administration could be easier using a larger needle. Most nurses (88%) found that during administration of rituximab SC it was easy to hold a conversation with the patient receiving the injection, with only

5% of nurses reporting that it felt unpleasant being close to the patient for the SC injection period of 6 minutes. Overall, nearly all nurses (95%) rated the overall experience with rituximab SC as 'positive' or 'very positive' and would recommend it to patients (95%). **Conclusions.** This survey revealed that nurses generally considered SC administration to be easy and would recommend it to patients. Nurses felt that the mode of administration allowed for conversation and easy interaction with the patient. Further improvements to the method of administration could be explored.

1750

A NOVEL AGENT, KL-21, HAS SIGNIFICANT ANTICANCER POTENTIAL ON CHRONIC LYMPHOCYTIC LEUKEMIA CELLS BUT NOT ON HEALTHY CELLS

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Background. Chronic lymphocytic leukemia (CLL) originates from antigen-stimulated mature B lymphocytes that either avoid death by external signals or die by apoptosis. Chromosomal translocations are rare in CLL and no specific mutations have been identified yet. Therefore, cytogenetic lesions are not likely to be a inducing factor in CLL. Chemotherapy, radiotherapy, biotherapy, transplantation of hematopoietic progenitors and monoclonal antibody immunotherapy are used for the treatment of CLL. However, these standart treatment methods are not sufficient to remove all CLL cells and have their own side effects. Therefore, developing new strategies or agents that could eliminate CLL cells has vital importance. **Aims.** The aim of this study to examine antiproliferative, cytostatic and apoptotic effects of KL-21 (produced by Naturin Natural Products Company, İzmir, Turkey) on 232B4 CLL cells. We also aimed to determine any adverse effects of KL-21 on healthy BEAS-2B human bronchial epithelial cells. **Methods.** Antiproliferative effects of KL-21 on 232B4 and BEAS-2B cells were determined by MTT cell proliferation assay. Apoptotic effects of KL-21 were determined by changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential, and Annexin-V staining by flow cytometry. Cytostatic effects of KL-21 were examined using DNase-free RNase and propidium iodine by flow cytometry. **Results.** 232B4 and BEAS-2B cells were treated with increasing concentrations of KL-21 (0. 0001-1 µg/µl) for 24, 48 and 72 hours and their proliferation was examined by MTT assay. There were significant decreases in proliferation of 232B4 CLL cells in a dose- and time dependent manner while KL-21 showed no cytotoxic effects on BEAS-2B healthy cells. IC50 values of KL-21 at 24, 48 and 72 hours were calculated from cell proliferation plots and found to be 0,2-, 0,1, and 0,08 µg/µl, respectively. Increasing concentrations of KL-21(0. 001- 1 µg/µl) increased caspase-3 enzyme activity, induced loss of MMP and triggered apoptosis significantly. There were 2-, 3-, 23-, 52- and 112% increases in caspase-3 enzyme activity; 31-, 45-, 260-, 312-, and 1340% increases in loss of MMP; and 16-, 23-, 28-, 77- and 228% increases in apoptotic cells in response to 0. 001, 0. 01, 0. 05, 0. 1 and 1 µg/µl KL-21, respectively, as compared to untreated 232B4 control. Treatment of 232B4 cells with KL-21 (0. 0001-1 µg/µl) for 48 hours resulted in a significant increase in G0/G1 and G2 phases while decreased S phase cell population as compared to untreated controls. **Summary and Conclusions.** Our results, in agreement with eachother, showed that KL-21 inhibits proliferation and cell cycle progression and induces apoptosis in a CLL cell specific way while it has no effect on healthy cells. Our data can suggest potential use of KL-21 for the treatment of CLL. The mechanisms of KL-21 induced apoptosis is under investigation in our laboratory both *in vitro* and *in invo*.

1751

MULTIPOTENT VDELTA2-NEGATIVE GAMMA-DELTA T-CELLS AFTER CMV-REACTIVATION IN ALLOGENEIC STEM CELL TRANSPLANTATION

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Background and Aims. Human cytomegalovirus (CMV) infections and relapse of disease remain major problems after allogeneic stem cell transplantation (allo-SCT), and adoptive transfer of antigen-specific αβT-cells has been proposed as a treatment strategy. However, transfer of antigen-specific αβT-cells is substantially hampered by their MHC-restriction and the fact that sufficient numbers of e. g. CMV-reactive αβT-cells can be exclusively isolated from CMV-positive donors. Great promise as an alternative strategy, with a potential use for very broad patient population, arises from the non-MHC-restricted target-recognition of cancer cells and virally-infected cells by γδT-cells. Therefore, we investigated the role of γδT-cells after allo-SCT and CMV infection. **Results and Conclusions.** An increase in selectively Vδ1-positive γδT-cells was observed in CMV-reactivating patients from CMV-positive conventional donors. Even more important, γδT-cell expansions were also observed after CMV-negative cordblood transplantation. However, in contrast to expansions from CMV-positive donors, γδT-cells from cordblood donors did not only include Vδ1-positive but also Vδ3-positive cells. Expanded Vδ2-negative γδT-cells were not only able to lyse CMV-infected fibroblasts, but also to partially mature dendritic cells (DCs) and to kill primary leukemic blasts. In order to investigate whether these different functions observed in γδT-cells which expand after CMV-infection are restricted to one cell type or to a diverse γδT-cell-repertoire which expands after CMV-infection, γδT-cells were further cloned by limiting dilution. CMV- and leukemia-reactivity were restricted to the same clonal population and separated from DC-maturation capacities of Vδ2-negative T-cells. Finally, γδT-cell receptor (TCR)-gene transfer experiments indicated that here-isolated Vδ2-negative γδTCRs selectively mediate anti-leukemia-reactivity and that activation of T-cells also depends on the presence of NKG2D. This makes CMV-reactive γδT-cells e. g. from cordblood donors as well as individual Vδ2-negative γδTCRs interesting candidates for adoptive immunotherapy strategies.

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THROMBOCYTOPENIA AS FIRST MANIFESTATION OF COELIAC DISEASE-CASE REPORT

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Introduction. Coeliac diseasse (CD) is the most frequent chronic misdiagnosed diseasse. For its development needs genetic predisposition and other factors. Incidence is 1/133 and between relatives 1/21. Besides gastroenterologic manifestations there are a big number of hematologic manifestations of CD (most frequent: anemia, thrombocitosys, IgA deficiency;rearly: thrombocytopenia, leukopenia, koagulation disorders, lymphomas and tromboembolic complications). We report a case of newdiagnosed CD with thrombocytopenia as only manifestation. **Case Report.** Female patient (pt), 55 years old, was examined because of thrombocytopenia- $43x10^9$ /l. Other parameters of blood cell count, biochemical analysis and virusological analisys where normal. Bone marrow aspirate showed megacariocytic thrombocytopenia. Indirect imunofluorescence (IIF) reveled presence of significant level of anti-endomisial antibodies (EMA). Because of thrombocytopenia at that moment gastrointestinal byopsi did not done. Gasteointestinal passage showed malapsortion. We started with gluten free diet without corticosteroid therapy. For one month platelet number normalised and remained normal untill nowdays- for 15 monts. After platelet normalisation gastrointestinal biopsy was done and confirmed CD. Pt was screened for Hashimoto thyreoiditis and diagnose was confirmed. She takes thyreosubstitutional therapy. As far as we know there are only three published cases like this. **Conclusions.** CD has many clinical manifestations and among population, very often, is a silent diseasse together with other autoimmune diseasses. It was showen that in some pts who were on gluten free diet hematological parameters where normalised, thus we recomend serological examination for CD in cases of autoimmune cytopenias, especialy in thrombocytopenia.

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IL-10 AND IL-17 GENE POLYMORPHISMS IN ADULT IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. In adults ITP is primarily characterized by increased platelet destruction, it is chronic, acquired and organ specific autoimmune disorder.

When the literature is analysed, many investigations about interleukin-10 and interleukin-17 gene polymorphisms in rheumatoid arthritis, Behçet's disease, Graves' disease, ulcerative colitis, asthma, atopic dermatitis, celiac disease in which autoimmune disorder is associated with their pathogenesis are seen and there are significant differences in some of them. In ITP which is an immune-mediated disorder there are a few study. **Aims.** In this study we aim to investigate IL-10 (-592 A/C) and IL-17 (A126G) gene polymorphisms and their serum levels in patients with ITP and control groups. **Methods.** We examined 50 patients with ITP and 50 healthy controls for IL-10 and IL-17 gene polymorphisms and 32 patients with ITP and 32 healthy controls with ELISA for plasma levels. **Results.** After the investigation of IL-10 (-592 A/C) and IL-17 (A126G) gene polymorphisms and their plasma levels in patients with ITP and control groups, no significant differences can be found in IL-10 (-592 A/C) and IL-17 (A126G) gene polymorphisms and IL-17 serum levels between patients with ITP and control groups. However IL-10 serum levels are significantly higher in patients groups compared to control groups (p:0,003). In addition we investigate the relationship between age, sex, treatment respond and platelet count in diagnosis in patients, IL-10 (-592 A/C) and IL-17 (A126G) gene polymorphisms and their plasma levels in patients with ITP but there is no significant differences between groups. **Conclusions.** The result of this study - there is no significant differences in IL-10 (-592 A/C) and IL-17 (A126G) gene polymorphisms and IL-17 serum levels between patients with ITP and control groups- demonstrates that there may be no contribution of them in developing ITP. IL-10 serum levels are significantly higher in patients groups compared to control groups. There are lots of contradictory results in IL-10 serum levels in ITP patients. Large prospective studies of cytokine gene polymorphisms are needed to determine the role they play in ITP.

1754

A NOVEL ADAMTS13 MUTATION IN THROMBOTIC THROMBOCYTOPENIC PURPURA PRESENTED IN AN OLD PATIENT

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Background. Thrombotic Thrombocytopenic Purpura (TTP) is a rare life-threatening disorder characterized by classic clinical pentad (thrombocytopenia, microangiopathic hemolytic anemia, fever, renal and neurologic abnormalities). It is associated with a deficiency of ADAMTS13 (a disintegrin and metallo-protease with thrombospondin type 1 motif, 13) that is responsible for cleaving large multimers of the von Willebrand factor. In adults, deficiency of ADAMTS13 are frequently caused from acquired production of antibodies to ADAMTS13. Inherited forms of deficiency is associated with mutations of the ADAMTS13 gene located on chromosome 9q34 and first acute episode of TTP usually presented in childhood. **Aims.** We herein report a case of TTP with new ADAMTS13 mutation whose first episode presented at the age of 61 and refractory to plasma exchange (PEX). **Case presentation and Methods.** A 61 year-old man was admitted to another university clinic in Turkey with headache, fever, hematuria and somnolonce. In examination, body temperature was 38,4ºC and widespread echymosis was noted. Laboratory was revealed thrombocytopenia (platelets 32x10^9 /L) and anemia (Hb 9,2 g/dl) with high creatinine (2,4 mg/dl) and LDH (930 U/L) levels. Schistocytes and decreased thrombocytes was noted in peripheral blood smear. With clinical and laboratory findings, patient was diagnosed as TTP and methylprednisolone 1 mg/kg and PEX for one volüme/day were started. After 22 cycle of PEX, thrombocytes was increased to 150x10^9 /L but LDH levels was continued to be high and schistocytes still found. Vincristine 2 mg was given and patient was accepted as refractory to PEX. Splenectomy was adviced but he refused. He admitted to our hematology clinic for evaluation after 1 month without PEX therapy. He had no any physical and neurological finding in our examination. Laboratory investigations were as follows; Platelets 123x10^9 /L, Hb 13,3 g/dl, Creatinine 0,51 mg/dl, LDH 427 U/L (NR 125-240 U/L). He was using methylprednisolone 32 mg/day. We performed ADAMTS13 gene analysis. The 7, 10, 11, 12 and 13 and 14th exons of ADAMTS13 gene were amplified on genomic DNA using HibriGen 2X PCR master mix (HibriGen Biotechnology R&D, Istanbul, TURKEY) according to the manufacturer's specifications. PCR products were genotyped by automatic DNA sequencing in a sequencer (ABI 3130xl, Applied Biosystems, Fosters City, USA) using dye terminator cycle sequencing kit (Applied Biosystems, Fosters City, USA). Heterozygous S553I mutation was found in exon 14 of the gene (Figure 1). Methylprednisolone tapered and patient closely followed without any treatment. After 4 months on follow-up, patient is in remission without any clinical and laboratory finding of TTP. **Conclusions.** We found a new ADAMTS13 gene mutation which caused TTP at the age of 61. To date, we did not find any report showing ADAMTS13 exon 14 S553I mutation. ADAMTS13 gene mutation must be considered in patients with TTP especially who is refractory to PEX and ADAMTS13 gene analysis must be performed without regarding any age group of patients before the decision of splenectomy.

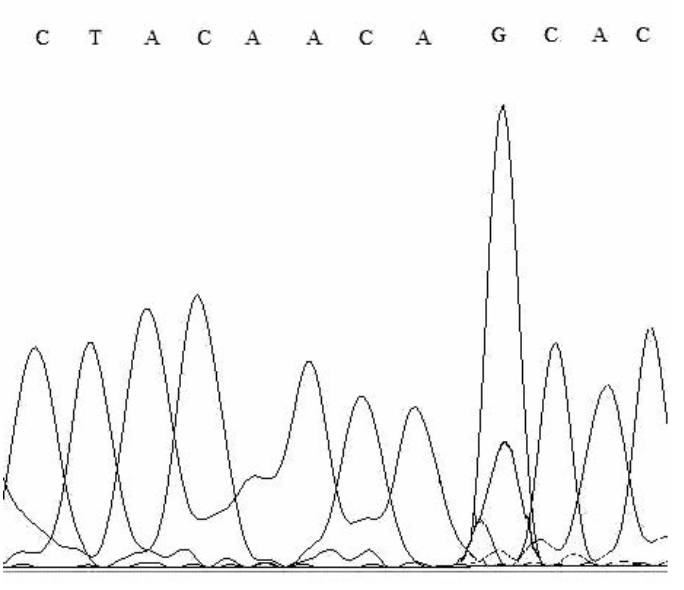

Figure 1. S553I heterozygous mutation in the ADAMTS13 gene.

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AN EXPERIENCE WITH LOW DOSE RITUXIMAB FOR THE TREATMENT OF PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA: A RETROSPECTIVE ANALYSIS OF SIX PATIENTS

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Background. In recent years, rituximab, at a standard dose of 375mg/m^2 has emerged as a valuable treatment option for patients with refractory immune thrombocytopenic purpura (ITP) inducing responses in up to 50% of patients. In fact, recently reported studies have suggested that a lower dose of rituximab (100 mg weekly for four weeks) may be an effective treatment regimen for refractory ITP. Such an approach has the potential to both reduce toxicities and also reduce healthcare associated costs. Rituximab is a chimeric monoclonal antibody which binds to the CD20 antigen on B cells and is highly effective in depletion of these cells. As a result of this depletion of B cells, a decrease in antiplatelet auto antibodies probably occurs, thus abrogating the thrombocytopenia. **Objectives.** To investigate the activity and safety of low dose rituximab in previously treated symptomatic ITP. **Methods.** Retrospective analysis of six patients with refractory ITP treated with a fixed dose regimen of rituximab (100mg weekly for 4 consecutive weeks). **Results.** Six patients were identified with chronic ITP. All patients had received two or more lines of prior treatment. Four patients had pre-treatment platelet counts below 20 x 10^9/L and two patients had platelet count of 20-30 x 10^9/L. Platelet responses were recorded 8-12 weeks post-treatment. One patient (male, age 46 years) had minimal but durable response (platelet 30-50 x 10^9/L) and remained asymptomatic. Second patient (female, age 76 years) showed no improvement in platelets. She required further treatment with pulse of dexamethasone to achieve stability (platelet 50 x 10^9/L). No response was observed in third patient (female, age 46 years) who required IVIG to maintain her platelets between 30-50 x 10^9/L. Fourth patient (male, age 42 years) failed to achieve any response (platelet < 10 x 10^9/L). He achieved complete response with romiplostim (platelets 121 x 10^9/L). Fifth patient had no response to low dose rituximab. She then received standard dose rituximab to which she had a partial response (platelet 56 x 10^9/L). Sixth patient (female, age 30 years) required no further treatment after low dose rituximab with platelet count 50-60 x 10^9/L. Thus 2 out of 6 patients had a minimal to partial response and were asymptomatic. Remaining four patients required further treatments after failure of low dose rituximab. **Conclusions.** This study shows that low dose rituximab produced significant overall response only in 2 out of 6 (33%) patients which is rather disappointing when compared to that seen with standard dose rituximab. Our experience with low-dose rituximab in this small group of patients suggests that further randomized trials are warranted involving a much larger number of patients with refractory ITP, preferably comparing standard with low dose rituximab before it is incorporated into standard treatment protocols.

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GENETIC ASSOCIATION OF INADEQUATE ANTIPLATELET EFFECTS OF ASPIRIN AND CLOPIDOGREL IN PATIENTS WITH CORONARY ARTERY DISEASE (CAD): A PRELIMINARY STUDY

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Background. Combination therapy with clopidogrel and low dose aspirin is the current standard of care in the management of patients with Coronary Artery Disease (CAD) including acute coronary syndromes (ACS). Between 4-30% of patients treated with conventional doses of clopidogrel show an inadequate platelet response. This may partly be done to underlying genetic diversity among individuals. Platelet resistance to aspirin is reported to affect <1% of patients as tested by light transmission aggregometry (LTA). To the best of our knowledge, no studies are available in the Indian population of genetic polymorphisms which may affect platelet response by these antiplatelet agents. **Aims.** To evaluate whether genetic polymorphisms are related to altered response to aspirin and clopidogrel. **Methods.** A total of 27 patients with CAD/ACS who were stable on dual anti platelet therapy (Clopidogrel 75 mg OD and aspirin 150 mg OD), were investigated along with age and sex matched 28 controls. Demographic and clinical data was collected on a predesigned clinical proforma. Platelet function testing by light transmission aggregometry was done with 4 agonists (ADP 2µM and 10µM, Epinephrine 5µM, Collagen 2µg/ml, Arachidonic Acid 0. 75mM) in each patient/control. After meta analysis the criteria employed for the Aspirin resistance was mean platelet aggregation ≥70% with 10µM ADP and ≥20% with 0. 75mM of Arachidonic acid. Aspirin semi responders were defined as those meeting only one of the above criteria. Clopidogrel resistance was defined as <10% decrease from the baseline in platelet aggregation in response to ADP 2 & 10µM. Semi responders were defined as 10-29% (<30%) decrease from the baseline. A baseline mean platelet aggregation obtained from 28 controls. Polymorphisms CYP2C19*2, CYP2C*3, CYP3A5*3 and PLA1/A2 were genotyped by PCR-RFLP. **Results.** Since arachidonic acid induced aggregation was adequately inhibited in all patients, no aspirin resistance was found. ADP induced aggregation, however was highly variable among patients. While no clopidogrel resistance was observed, 13(48%) patients were semi responders to clopidogrel. had higher frequency of the *2 Allele of CYP2C19 as compared to responders (p=0. 004). The mutant homozygous and heterozygous genotypes (*2/*2 or *2/*1) of CYP2C19 was found to be significantly higher in clopidogrel semi responders than in responders patients (100% vs 35. 7%, p=0. 0006 respectively). Also ADP (10µl) showed significantly reduced platelet inhibition in patients with mutant homozygous/ heterozygous genotypes ((*2/*2 or *2/*1) of CYP2C19 as compared to patients with wild type (*1/*1) genotype (p=0. 0001). **Conclusions.** There was significantly higher frequency of mutant genotype of CYP2C19 in clopidogrel semi responders as compared to responders. The clopidogrel response to antiplatelet drugs was inadequate (semi responders) in those with mutant genotypes. These preliminary findings suggest that polymorphisms may be associated with inadequate response to antiplatelet drugs.

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THROMBOCYTOPENIA IN NEONATES: CAUSES AND OUTCOMES

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Neonatal thrombocytopenia is defined as a platelet count <150,000/mm³ in any neonate of a viable gestational age. Thrombocytopenia is one of the most common hematologic abnormality in the neonatal period. This study which is purposed to decrease prevalence of thrombocytopenia by eliminating preventable factors and to determine the treatment and clinical outcomes of neonates with thrombocytopenia, in the neonates admitted to neonatal service and neonatal intensive care unit, is designed as a retrospective study. In 3515 neonate the thrombocytopenia prevalence is found 3. 8 % of all neonates (1. 3 % in terms, 12% in preterms) and 12 % in those admitted to the neonatal intensive care unit. Sepsis and IUGR are found as the most common causes of thrombocytopenia. The analysis of the prevalance of thrombocytopenia according to years revealed that the highest prevalence was in the year 2008 by 5. 3 %, and the lowest prevalance was in the year 2011 by 2. 4%. When the caues of thrombocytopenia were analysed, it is seen that sepsis was the most common reason between years 2007-2009, whereas IUGR took place in 2010. On the other hand, thrombocytopenia caused by metabolic disorders, drugs and asfixia was more common in 2011. The median day of determination of thrombocytopenia was found as 1st posnatal day, median nadir platelet count was found as 6th day, median improvement day was found as 10th day. Median platelet count was 50,000/mm³. Severe thrombocytopenia was found in 26 % of neonates and %11 of thrombocytopenic neonates had major hemorrhage. Intracranial hemorrhage ratio was 5. 9 % and all of these patients were preterm. The incidence of hemorrhage increased with severity of thrombocytopenia. Severe thrombocytopenia was significantly higher in mechanically ventilated neonates. Platelet transfusion was given to 33% of neonates and the mortality rate was increased in these neonates. Thrombocytopenia improved in 93 % of patients and persisted in %4 of patients. Death occurred in 3% of neonates with thrombocytopenia but the causes of death in those patients were not attributable to thrombocytopenia. In conclusion, we found that the prevalance of thrombocytopenia decreased significantly during recent years and the distribution of causes of thrombocytopenia according to years changed in our neonatal service and neonatal intensive care unit. This study shows that rate of thrombocytopenia can be lowered by eliminating prevantable factors of thrombocytopenia in neonates and as a result, complications and risks of thrombocytopenia and platelet transfusion can be decreased.

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DEVIATIONS FROM NORMAL VALUES OF LEUKOCYTE AND ERYTHROBLAST PARAMETERS IN COMPLETE BLOOD COUNT IS A MESSENGER FOR PLATELET ABNORMALITIES

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Background. Automated blood cell counters have undergone a formidable technological evolution owing to the introduction of new physical principles for cellular analysis and the progressive evolution of software. The results have been an improvement in analytic efficiency and an increase in information provided with new parameters. **Aims.** In this case report, we imply the incompatibilities between uncorrected leukocyte count (UWBC), leukocyte count (WBC), and erythroblast count (NRBC) might be predictors for morphological and numerical abnormalities of platelets. **Methods.** A 61 year old male patient had the diagnosis of ''diffuse large B-cell lymphoma'' six years ago and after chemotherapy was still in remission. He was hospitalized for high fever, fatigue, acute renal failure and bibasilar crepitant rales. Complete blood count revealed UWBC 63. 5 x10^9/l, WBC 22. 1 x10^9/l, NRBC 21. 4 x10^9/l, and platelets 197 x10^9/l. On peripheral blood smear examination we detected 5% neutrophils, 22% band forms, 61% metamyelocytes, 5% myelocytes, 1% promyelocyte, 2% myeloblasts, 2% lymphocytes and 2% eosinophils. We also detected rare erythroblasts and large platelets with profuse platelet clumps. Routine biochemical analysis revealed high fasting glucose, blood urea nitrogen, creatinine, SGOT, alkaline phosphatase, direct and indirect bilirubin, albumin, and lactate dehydrogenase. Erythrocyte sedimentation rate was 100 mm/hour, and serum ferritin was 2 944 ng/ml. High resolution computed tomography of thorax revealed bilateral diffuse infiltrations, nodular opacities, right pleural effusion, and mediastinal lymphadenopathies.

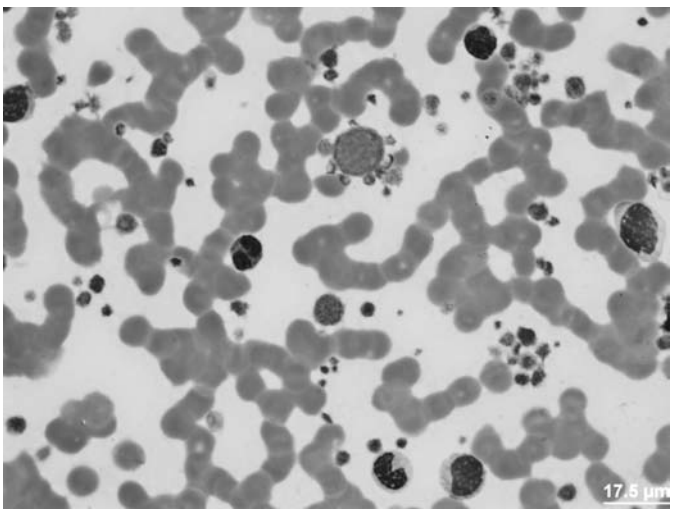

Figure 1. Peripherall blood smear.

Results. Clarithromycin and imipenem/cilastatin were administered for probable diagnosis of pneumonia. Bone marrow examination had myeloid hyperplasia but nothing significant else. No endobronchial mass was detected in bronchoscopy, but mucopurulent secretion was present in right upper and lower lobes. Biopsy reports were non-neoplastic bronchial mucosa epithelium. Sputum, blood, urine cultures, sputum mycobacterial examination and serum galac-

tomannan antigen were negative. After general condition, fever, acute renal failure, signs and symptoms relieved, he was discharged. **Conclusions.** When we subtracted WBC and NRBC from UWBC (=20. 0 x10^9/l), a significant cell group was composed of big platelets. It is probable that this ratio is higher than calculated. Rare erythroblasts in peripheral blood smear with high NRBC values support the idea of large platelets as cellular origin. In fact, peripheral blood smear revealed large, profuse platelet clumps contradictory to platelet count (Figure 1). We conclude that complete blood counts should be examined carefully, despite the essential role of automation in the modern hematology laboratory, microscopic control of pathologic samples (peripheral blood smear) remains indispensable, so much so that in certain cases, it alone is diagnostic.

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ITP SUCCESSFUL TREATMENT WITH ELTROMBOPAG FOLLOWING MULTI-THERAPY FAILURE (INCLUDING ROMIPLOSTIM)

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Immune thrombocytopenia (ITP) is an heterogeneous autoimmune disorder characterized by low blood count of PLTs with o without muco-cutaneous bleedings. Recent studies have found an increased incidence and prevalence of ITP in elderly people and the presence of additional co-morbidities makes the choice of the treatment more challenging. TPO receptor agonists have been recently licensed for patients who underwent splenectomy or when the operation is contra-indicated. Randomised trials report high response rate in refractory post-splenectomy patients, but a proportion still remains thrombocytopenic to both the drugs and no studies have described results after the switch from a TPO to another. Case report: this 84-year-old man was admitted to our hospital in January 2008 because of the haemorrhagic syndrome characterized by petecchiae, ecchymoses and epistaxis. Blood tests demonstrated severe thrombocytopenia with platelet count of 3x10^6/ml, WBC 12x10^6/ml and Hb of 12. 6 g/l. A bone marrow aspirate was consistent with a diagnosis of ITP, revealing an increased number of megakaryocytes. 1 g/kg/die I. V. Ig for two days plus oral prednisone at the dose of 1 mg/kg/d were administered without any platelet response. Over the following weeks the patient experienced further muco-cutaneos bleedings and anaemia, which required blood and platelets transfusions. We considered splenectomy no feasible at that moment and the patient started rituximab 175 mg / weekly for four weeks, without any result. Afterwards when the patient experienced another hemorrhagic event he was treated with I. V. Ig and platelet transfusions again until he was then enrolled (after 14 months from diagnosis) in the experimental trial AMG 531 (Romiplostim) and although he was treated up to the maximum recommended dosage of 10 µg/kg, he did not obtain and PLTs increase. The patient after a period of stable low platelets count (10 x 10^6/ml) experienced a new major GI hemorrhagic event that required bloodand platelets transfusions. So having no other chance, 17 months later the patient underwent splenectomy without serious adverse events but unfortunately without any PLTs increase. Concurrently with another major hemorrhagic event, due to a bladder papilloma, that required blood transfusions, the patient started treatment with eltrombopag(in the meanwhile licensed for ITP in Italy) and after four weeks at the dose of 50 mg/day we observed a platelets count over 100x10^6/ml which is maintained stable until now (20th week). No increase of liver enzymes or other adverse events were registered. Both eltrombopag and romiplostim are currently licensed for treatment in adults with chronic ITP who have failed to corticosteroids, immunoglobulins and splenectomy. Romiplostim and Eltrombopag both take a new approach to treat ITP, instead of reducing platelets destruction, these drug stimulate thombopoiesis, with a different cellular mechanism. Because both drugs take nearly 30% failure, the possibility to switch from a drug to another may increase the chance of success also in the subgroup of the very multi-therapy resistant patients.

1760

SUCCESSFUL MANAGEMENT OF A SMALL INFANT WITH KASABACH MERRITT PHENOMENON USING VINCRISTINE: A CASE REPORT

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Introduction. Kasabach-Merritt Phenomenon (KMP) is characterized by profound thrombocytopenia, microangiopathic hemolytic anemia, a consumptive coagulopathy and an enlarging vascular lesion. The syndrome develops in infancy and is associated with a high morbidity and mortality rate. **Aims.** To present a case report of successful management of KMP in an infant using vincristine, after the failure of combination of corticosteroids and propanolol. **Case presentation.** Newborn female, of uncomplicated twin pregnancy, was born with a giant hemangioma located in the right thigh. From the first hours of life she was treated with combination of corticosteroids and propanolol, however, there was no improvement in the size of the lesion and severe, persistent thrombocytopenia, anemia and hypofibrinogenaemia developed. Until the 45th day of life, despite therapy, the tumor showed dramatic increase in size, with occupation of the whole limb and the major part of the abdomen. Due to extremely low values of platelets, hemoglobin and fibrinogen, the neonate was treated with daily administration of red blood cells, platelets and cryoprecipitate. The resistance to administered treatment and the risk of serious bleeding led to the decision of starting vincristine the 45th day of life. The drug was administered at a dose of 0. 05 mg/kg in weekly intervals. During the first week of therapy hematological parameters improved rapidly and on the second week the infant had no need for blood products. The third week the number of platelets and fibrinogen were normal for the infant's age, the size of the tumor had shrunk dramatically (reduction in tumor perimeter6 cm) and it's location was limited in the thigh. Until today, the infant has received 10 doses of vincristine, without experiencing any side effects and with continuous improvement of the clinical picture. **Conclusions.** Administration of vincristine proved to be safe and effective, where other drugs had failed in managing a small infant with Kassabach Meritt phenomenon.

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PLATELET MORPHOLOGIC INDICES IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM

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Background. Primary hyperparathyroidism (P-HPT) is characterized by the autonomous production of parathyroid hormone (PTH) resulting in hypercalcemia which represents the most common cause of hypercalcemia in the outpatient setting, in patients without an underlying malignancy. Nowadays, most patients are asymptomatic and identified incidentally on routine serology. Patients with P-HPT present an increased risk for cardiovascular disease and hypertension. The exact role of P-HPT on platelet function remains largely unknown. Platelet parameters and especially mean platelet volume (MPV), an important determinant of platelet function, morphology and activation, constitutes a novel emerging risk factor for atherosclerosis and its complications such as coronary heart disease. It has been suggested that P-HPT could represent a risk factor for cardiovascular disease, especially coronary heart disease. MPV, platelet distribution width (PDW), platelet count (PLT) have not been studied in depth in P-HPT. **Aims.** The purpose of the present study was to compare the platelet count as well as the platelet indices MPV and PDW in asymptomatic patients with P-HPT and in age-, gender and body mass index (BMI)-matched healthy subjects as well as to investigate whether P-HPT may present a predictive significance in the determination of platelet morphology. **Methods.** In a cross-sectional study between 2007 and 2011, we have evaluated eighteen patients with P-HPT due to a parathyroid adenoma, prior to any therapeutic intervention (parathyroid surgery) (15 women and 3 men) with a mean age: 49. 05 ± 8. 1 years (range: 33-64 years) and a double number of healthy subjects (30 women and 6 men) with a mean age: 51. 02 ± 9. 3 years (range: 32-66 years). Healthy subjects were matched (2 controls to one patient) on gender, age (±5 years), body mass index (± 1. 5 kg/m^2) and year/month of diagnosis (±1 month). None of the subjects (patients and controls) presented any infectious and neoplastic conditions, diabetes mellitus, hypertension and dyslipidemia. To assess thrombopoiesis, we have determined platelet indices using the Sysmex 9000 analyzer. Statistical analysis of data was performed with IBM-SPSS® statistical package version 20 for Windowssoftware. **Results.** Cases with P-HPT presented significantly higher MPV (mean ± SD: 12. 2 fL ± 0. 44) and PDW (mean ± SD: 15. 76 % ± 0. 9) than controls (mean MPV ± SD: 10. 77 fL ± 0. 78, p<0. 001 and mean PDW ± SD: 13. 69 % ± 1. 21, p<0. 001). Moreover, patients with P-HPT exhibited a higher number of platelets per mm^3 than healthy controls (mean PLT in patients: 298 x 10^3/mm^3 ± 33 versus mean PLT in controls 263 x 10^3/mm^3 ± 37, p=0. 002). In a linear regression model, adjusting for age, gender and BMI, the presence of P-HPT was the most significant determinant of MPV and PDW levels (p=0. 001). **Conclusions.** These results suggest that patients with P-HPT tend to present an augmented platelet size. Elevated platelet activation could contribute to an increased risk of cardiovascular complications observed in P-HPT. Further larger studies are needed in order to evaluate the contribution of platelet parameters to the risk for cardiovascular complications in patients with P-HPT.

1762

EFFICACY EVALUATION OF RITUXIMAB THERAPY IN IDIOPATHIC THROMBOCYTOPENIC PURPURA REFRACTORY TO CORTICOSTEROIDS TREATMENT

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Background. The mainstay of initial therapy of idiopathic thrombocytopenic purpura (ITP) has included corticosteroids, but in steroid-refractory patients splenectomy is the treatment of choice. In the majority of cases splenectomy is associated with high risk of hemorrhagic complications, and in 30% cases the desirable results are not achieved. Those whom splenectomy and steroid therapy fail to cure are treated empirically and in most cases durable remissions are not attained. Due to this fact,researches of prognostic criteria for the therapy with rituximab as well as developing the definitive courses are essential and crucial for the practical medicine. **Aims.** The goal of the study is to analyze the efficacy of rituximab treatment in patients suffering from ITP. **Methods.** 32 patients were included into the assay. Among them: 14 patents received the therapy with rituximab following failed significant response to splenectomy performed on an average 3,8 years ago; 18 patients were treated with rituximab after previous three ineffective courses with steroids without splenectomy. All the patients received rituximab at a dose of 375 mg/m^2 intravenouslyonce weekly for 4 weeks. 3 patients who had splenectomy performed in the past received 4 doses of Nplate during the chemotherapy course. The follow up continued for 16 months. In view of development of severe trombocytopenia in all the patients before the rituximab therapy, IgG products were administered intravenously for 5 days. **Results.** The positive effect of rituximab therapy was observed in 6 patients (43%) from splenectomy group. After immunoglobulin G infusions the platelets counts rose by 32,5% before the administration of rituximab. 8 patients from splenectomy group showed no effect neither from immunoglobulin, nor from rituximab therapy. Among the group of patients without previous splenectomy the platelets count reached the normal values in 78% (14 patients) within 2,1 months. All these patients had increase in platelets count by 44. 2% after injections of immunoglobulins G. Initially, there was an elevated level of immunoglobulins G by 74,7% compared to normal values and decrease of relative count of CD20 lymphocytes in all the patients who responded positively to the therapy with IgG. **Conclusions.** Rituximab can be used as a first-line therapy for ITP in patients who had splenectomy performed as well as in patients without surgery. To assess the potential rituximab therapy efficacy the following prognostic criteria can be used: the elevated level of IgG in blood without evidence of acute infection; positive response to IgG products treatment.

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IMMUNE THROMBOCYTOPENIC PURPURA OF CHILDHOOD WHAT IS THE BEST TREATMENT?

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Background. Immune thrombocytopenic purpura (ITP) is an acute self-limited disease of childhood, resolving mostly within 6 months with or without treatment. Bleeding is usually less than expected and severe hemorrhage is seen in less than 1% of children. Treatment options are steroids, IVIG or anti-D Ig. **Aims.** To share our experience of 32 years of different treatment modalities used for ITP. **Methods.** Fivehundred-fortyone children (M/F: 1. 1; mean age: 5. 32 ± 3. 67 years) with ITP, referred to our unit between 1978 and 2010 were evaluated retrospectively with regard to their clinical, laboratory features, etiological factors and different therapeutic modalities used. **Results.** Nine percent of the cases were less than 1 year old. In 30% of the patients, there was a recent history of infection and/or vaccination. Most frequent symptoms were echymoses and petechiae, severe central nervous system bleeding was seen only in 0. 8% of the patients. Mean initial platelet count was 19. 353/mm3. The 491 acute ITP patients were treated initially as follows; 134 (27%) by IVIG, 133 (27%) by high dose methylprednisolone (HDMP), 101 (20%) by standart dose steroids (SDP), 13 (2%) by anti-D Ig. Twent-two percent were followed without treatment. When the response to different treatment modalities was evaluated; response rate was 84. 2% in patients treated by HDMP, 77. 6% treated by IVIG and 89. 1% treated by SDP. The response duration was 16. 8 ± 13. 0 days, 3. 8 ± 3. 3 days and 3. 0 ± 2. 1 days in patients treated with SDP, HDMP and IVIG respectively. **Conclusions.** IVIG, standart and high dose steroids are all successful treatment options with similar response rates. The duration of response is slower in patients treated with SDP compared to IVIG and HDMP.

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IMMUNE THROMBOCYTOPENIA IN THE ELDERLY: CLINICAL COURSE IN 525 PATIENTS FROM A SINGLE CENTER IN CHINA

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Background. Immune thrombocytopenia (ITP), which is often diagnosed in the elderly, is a hematologic disorder characterized by thrombocytopenia induced by autoimmune mechanism. **Aims.** In this retrospective study, we evaluated the clinical features, the risk of bleeding and the response to various treatment options in elderly ITP patients (age≥60 yr) at our center from 1980 to 2009. **Methods.** We retrospectively analyzed the medical records of 525 aged Immune thrombocytopenia (ITP) patients (325 females and 200 males) from 1980 to 2009. The median age was 66 years (range 60-91 years) and the median platelet count was 20×109/L (range 1-95×109/L) at diagnosis. The median duration of follow-up was 35 months (range 1-253 months). **Results.** At diagnosis, 461 patients (87. 8%) had symptoms or signs of bleeding. The risk of bleeding was significantly related to platelet count (OR: 0. 956; 95% CI: 0. 944-0. 967). Among all the 525 patients, we found 328 patients (62. 5%) had comorbidities. One hundred and forty-four patients (27. 4%) received no treatment, of which 22 cases achieved CR spontaneously. The first-line treatment included corticosteroids and/or intravenous immunoglobulin. The total response rate was 58. 9% (165 of 280 patients), and this rate was not significantly related to platelet count, sex, bleeding signs and age (the 60-74-year group vs. the 75-91-year group). Only fourteen patients (2. 7%) underwent splenectomy. In the evaluable 23 patients who died during follow up, seven deaths were considered to be directly attributable to hemorrhage induced by thrombocytopenia. **Conclusions.** In conclusion, there are more females in the ITP patients over 60 years of age. The risk of bleeding is quite high in elderly ITP patients, but life-threatening bleeding events rarely occur. This retrospective review represents the largest collection of elderly patients diagnosed with ITP in China in a single center.

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DISEASE PRESENTATION AND MANAGEMENT PRACTICES OF CHILDHOOD IMMUNE THROMBOCYTOPENIC PURPURA

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Background. ITP is the most common acquired bleeding disorder of childhood, accompanied by many controversies in diagnosis and management. It is a heterogeneous bleeding disorder with a diverse natural history. Treatment differs worldwide in terms of when to treat, what treatment to use and the need for hospitalization. **Aims.** We aimed to report our experience with such heterogenous disease through describing the detailed epidemiological features, clinical presentations, disease course and treatment modalities and to evaluate the influence of different factors on outcome, course and development of SLE in children with ITP. **Methods.** Retrospective study of the medical records of 202 children with ITP who were diagnosed, treated and followed up at pediatric hematology unit of Zagazig university hospital, between January 2005 and December 2010. **Results.** Mean age of patients was 4. 56 ± 2. 9 years with male to female ratio of 1. 1:1. Mean initial platelet count was 17. 2 ±10. 6 X 10^9/l . Cutaneous bleeding alone was found in 61% of patients, epistaxis in 23. 3%, bleeding gums in 15. 8%, urogenital bleeding in 3% and gastrointestinal bleeding in 2. 5% of patients. Intracranial hemorrhage was reported in only two patients during the study period. 20. 3% of patients were managed conservatively with spontaneous recovery. No significant difference in platelet counts at days 3,7 and 14 after treatment between patients receiving steroids only, IVIG only, or both steroids and IVIG. 17. 8% of patients developed chronic ITP and 3% developed SLE. Predictors of Chronicity were female gender, older age and ANA positivity. Anti D, Splenectomy, Azathioprine, Danazole and Rituximab were tried with varying results. **Summary and Conclusions.** ITP is a self limiting disorder with 80% of patients having acute disease and recovery . Steroids and IVIG are equally effective as a first line therapy. Despite of the good initial response to Anti D, Danazole, Azathioprine and splenectomy, many patients experienced relapse. Large studies about novel therapies such as Rituximab are needed. ANA test is a useful screening test that predicts initial response to steroid therapy, development of chronicity and risk of progression to SLE.

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A COMPARATIVE STUDY OF PLATELET (PLT), AND THEIR AVERAGE VOLUME (MPV), IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND ON HEALTH PEOPLE

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Background and Aims. To evaluate both the number and the average volume of platelets in diabetes mellitus patients, and compared our results with those of healthy, since the average volume of platelets is directly related to the activity, which as we all know contribute the onset of cardiovascular diseases. **Methods.** A total of 106 cases studied diabetes mellitus(DM) patients (61 males and 45 females, average age 66. 8 years), and 85 healthy persons (47 males and 38 females) approximately average age 70. 2 years, who examined in the outpatient hypertension and atherosclerosis department of the Psychiatric Hospital of Thessaloniki. In all recorded platelet count (PLT) and average volume (MPV), while in 78 of the 106 patients with (DM) were measured additionally prices haemoglobin glycosylated (HbA1c), as determined by the automatic biochemical analyzer using the chemiluminescence. **Results.** Regarding the number of platelets was observed between the two groups no statistically significant difference (p> 0,05). Throughout, the MPV was statistically highly increased (p <0,001) in the patients with compared with (DM) the healthy group. Specifically, the patients had (DM) average MPV 12,6 fl (79 by 106, a rate 74. 5% were abnormal), while for healthy controls was 8,6 fl. (Only 15 of 85, ie 17. 6% rate, showed elevated MPV). Indeed, further investigation showed correlation of MPV (13,9 fl) with cardiovascular events (acute myocardial infarction and stroke in 16 and 12 cases from the total of 106 (DM), respectively), while the levels of the MPV is not proved to be related, nor sex, and even with levels of HbA1c. **Conclusions.** It turns out therefore that the MPV in patients with type 2 (DM) is significantly increased compared to the health general population, which implies an additional and independent cardiovascular risk factor. Therefore, in (DM) group, should do, an regular monitoring for cardiovascular disease, but without forgetting, and frequent monitoring of blood profile.

1767

THE MORPHOLOGICAL CHANGES OF PLATELETS CAN ABERRANTLY INCREASE THE VALUE OF IMMATURE PLATELET FRACTION DURING COLD STORAGE

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Background. Reticulated platelet percentage (RP%) is a useful marker of thrombopoiesis, and helpful for differential diagnosis in thrombocytopenic patients. For measurement of RP%, immature platelet fraction (IPF%) is determined using the automated hematology analyzer in a simple and reproducible way between laboratories, although it has some limitations. The IPF% rises during storage at 4˚C, and the aberrant increase is also observed in some myelodysplastic syndrome patients especially with karyotypic abnormalities. However, the reason has still remained to be clarified. **Aims.** We investigated the shape change and activation status of platelets during cold storage in order to elucidate the mechanisms of the aberrant increase of IPF. **Methods.** The IPF and IPF% were determined using Sysmex XE-2100 automatic hematology analyzer in the blood samples of healthy donors before and after storage at 4˚C for 24 hr. Platelet count and other parameters of platelet, such as MPV, PDW and P-LCR were measured simultaneously. Phosphorylation of myosin light chain was also examined to estimate activation status. The morphological changes of platelets were also examined on the blood film. **Results.** The IPF and IPF% was significantly increased during storage, accompanied with the shape change and myosin phosphorylation, which indicated platelet activation. Morphologically, each platelet increased in size with fewer granules probably due to degranulation, and a couple of platelets stuck to each other to form a few small clumps. For further investigation, we examined the blood samples with previously recognized mild EDTA-induced platelet aggregation. The IPF% significantly increased in EDTA-anticoagulated blood compared to other anticoagulants such as heparin. **Conclusions.** Although the mechanisms seem to be complicated, the morphological changes such as degranulation and enlargement of platelets can play a significant role in aberrant increase of IPF% during cold storage.

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EFFECT OF ANTI-CD20 MONOCLONAL ANTIBODY (RITUXIMAB) IN IMMUNE THROMBOCYTOPENIA. EXPERIENCE IN A PERUVIAN HOSPITAL

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Objective. We describe the response to Rituximab in immune thrombocytopenia in adult patients of Rebagliati Hospital which belongs to social security system. **Materials and Methods.** is a retrospective, descriptive and observational study. We reviewed the medical records of adult patients with immune thrombocytopenia who received Rituximab after failure to reach or maintain Response with at least one line of treatment, from 2005 until December 2010. According to the consensus of the international working group on ITP, it was considered: i. Response: platelet count > 30 000 and at least double the initial platelet count, ii. Complete Response: platelet count> 100 000 and no signs of bleeding. **Results.** A total of 24 courses of Rituximab 375mg/m2 each week for 4 weeks were assessed, mean age of patients was 48. 6 ± 17. 6 years (range, 25 - 80) with a male/female ratio of 1/3, and a mean of previous treatments of 2. 8 ± 1. 46 (range, 1 - 6). Response was reached after 18 courses (75%), in a mean time of 11. 9 ± 11. 8 weeks (range, 0. 7 - 37. 4), mean duration of response of 15. 9 ± 12. 7 months (range, 3. 3 - 55. 3 months) and 12 patients still remain in response until last follow up (mean 18. 6 ± 14. 3 months; range, 3. 3 - 55. 3 months). Similarly, Complete Response was reached in 14 of 23 courses evaluated (60%) in a mean time of 17 ± 15. 9 weeks (range, 0. 7 - 62. 3 weeks), with a mean duration of complete response of 10. 1 ± 7. 3 months (range, 2. 3 - 25. 2 months), 5 patients still remain in Complete Response until last follow up (mean, 16. 9 ± 8 months; range, 8 - 29 months). We found that splenectomized patients has a greater chance of maintain the response obtained compared with non splenectomized patient (8 vs 4 patient; OR: 4), and similar results for Complete Response (4 vs 1 patient; OR: 14). **Conclusions.** Rituximab is a good alternative in patients with ITP who failed at least one line of treatment.

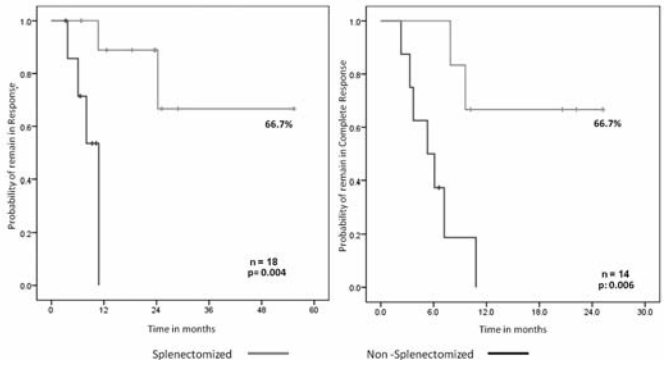

Figure 1. Kaplan-Meier estimates of probability for remain in response, and complete response in ITP patients after use of Rituximab by splenectomized status. Rebagliati Hospital - 2011.

1769

EFFICACY AND SAFETY OF ELTROMBOPAG TREATMENT FOR PATIENTS WITH REFRACTORY OR RELAPSED CHRONIC IMMUNE HROMOCYTOPENIA (ITP)

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Background. Chronic immune thrombocytopenia (ITP) is an autoimmune disease characterized by low platelet counts due to increased platelet destruction and suboptimal platelet production. Eltrombopag, a non-peptide oral platelet growth factor, increased platelet counts in adults with chronic ITP . It has been revealed that it stimulates TPO-dependent cell lines via JAK2 and STAT-signaling pathways to proliferation, megakaryocyte differentiation and platelet production. **Aims.** To evaluate efficacy and safety of eltrombopag treatment for patients with refactory or relapsed chronic ITP. **Methods.** we studied 43 patients with chronis ITP hospitalized in the Clinic of Hematology of Craiova (Romania) between 2009-2011 (informed consent obtained). All patients were initially treated with corticosteroids (prednisone or high dose dexamethasone) and 8 of them were splenectomized. Nine patients with refractory or relapsed chronic ITP and platelet counts ≤ 30. 000/μl were treated with eltrombopag at a starting dose of 50 mg daily. At patients who did not respond to eltrombopag 50 mg after 30 days the dose was increased to 75 mg daily. Patients who achieved platelet

counts ≥ 150. 000/ µl on eltrombopag 50 mg had their dose reduced to 25 mg daily. Patients with a prior history of arterial or venous thrombosis and known risk factors for thrombosis and patients with a history of cardiovascular disease were excluded from the eltrombopag treatment. **Results.** Six patients responded at eltrombopag 50 mg daily (platelet counts ≥ 150. 000/ µl) after the first month of treatment and dose has been reduced to 25 mg daily. Two patients responded after two months of treatment at a dose of 50 mg daily and one patient was refractory to this treatment after three months of treatment at a dose of 75 mg daily and was splenectomized. Headache was observed in two patients, one patient developed an increase in ALT and hyperbilirubinemia; no patient had thromboembolic events. **Conclusions.** Eltrombopag treatment at the initially dose of 50 mg daily was efficient and safe in 88% cases; a single patient was reluctant and splenectomized. Because the group was small in size statistical significance was not achieved. In the future, we are planning a study on a larger group of patients in order to clarify the results of eltrombopag therapy in chronic ITP.

1770

SIMVASTATIN AND AMLODIPINE INDUCED THROMBOCYTOPENIA IN THE SAME PATIENT: A DOUBLE TROUBLE

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Background. Drug induced thrombocytopenia (DITP) is a serious but rare side-effect of a wide range of medications. DITP is often misdiagnosed as acute immune thrombocytopenic purpura (ITP). The aetiology of DITP is complex and at least six distinct mechanisms have been identified by which drug-induced antibodies can promote platelet destruction. Amlodipine and simvastatin have rarely been implicated in DITP. Amlodipine is a dihydropyridine calcium-channel antagonist that reduces the influx of extracellular calcium into cardiac and vascular smooth muscle cells via L-type calcium channels. Simvastatin is a prodrug metabolised in the liver to form simvastatin acid, an inhibitor of HMG-CoA reductase. Both agents are recommended in hypertensive diabetic patients. In a systematic review of case reports two cases of amlodipine-induced DITP and four cases of simvastatin-induced DITP have been reported so far. **Aims and Methods.** We present a patient who exhibited in a short period three successive episodes of DITP, first two on amlodipine (induction and rechallange), and the third on simvastatin. **Results.** A 78-year-old female with type 2 diabetes mellitus, dyslipidemia, hypertension, stabile angina pectoris, bronchial asthma, and chronic nephropathy was referred to the hospital due to 3-day history of easy bruising, signs of skin and wet purpura and isolated severe thrombocytopenia of $1x10^9$/L. She had been on long-term treatment with a number of drugs, and also received a short course of amlodipine, 2 weeks before admission. Serum creatinine and blood urea nitrogen were 328 µmol/l and 15. 0 mmol/l respectively, cholesterol was 6. 7 mmol/L, LDL-cholecterol 4. 5 mmol/L and triglicerides were 2. 8 mmol/L while all other analyses, including autoimmune screens, viral and coagulation tests were within normal ranges. Peripheral blood smear ruled out psudothrombocytopenia and bone marrow aspirate was unremarkable. Standard treatment for ITP (prednisolone 1mg/kg) was initiated with no improvement in platelet count in following days. Than amlodipine, being the last introduced drug, was discontinued. Platelet count showed sustained increase to $126x10^9$/L on day 7 when corticosteroids were fully discontinued. She was discharged from hospital on day 17 with platelet counts of 165x109/L. Simvastatin, which was taken for several years and was discontinued for three months, was reinduced due to dyslipidemia by endocrinologist on d27. Immediately after ingestion of first dose she felt generalized pruritus and referred to hospital with generalized urticaria where drop in platelet count to $98x10^9$/L was registered. Simvastatin was ceased, and platelets after further decrease to $65x10^9$/L on day 30, in following days showed sustained rose to $226x10^9$/L and remained stable in next four months. Than she took by accident one tablet of amlodipine (10mg) causing immediate drop in platelet count to $35x10^9$/L, so fulfilling Georges clinical criteria for definitive level of evidence for DITP. **Conclusions.** As of our knowledge, this is the first case report on successive DITPs on two widely prescribed drugs. Concomitant administration of calcium-channel blockers and statins has multiple beneficial effects on cardiovascular system, but on the other hand may enhances a chance for drug induced hypersensitivity and makes the identification of causing drug or drugs almost impossible.

1771

THROMBOPOIETIN RECEPTOR AGONIST IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA: CLINICAL EXPERIENCE

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Background. Inmune thrombocytopenic (ITP) in adults is a chronic autoimmune disorder characterized by low platelet counts (PC) and mucocutaneous bleeding due to accelerated platelet destruccion and suboptimum platelet production. Many treatments have focused on reduction of platelet destruction; nowadays the thrombopoietin-receptor agonist (Tpo-RA), Romiplostin and Eltrombopag, are new treatment modalities and exerting their effect by stimulating platelet production, alone or in combination with existing therapies, reducing the risk of bleeding. **Aims.** To describe our experience with the Tpo-RA therapy in patients with chronic ITP: platelet counts, bleeding clinical, decrease or discontinuation of concomitant ITP drugs, rescue medication in emergency**,** incidence and severity of adverse events and treatment-related mortality. **Patients and Methods.** Retrospectively we have analyzed 15 patients with chronic ITP in our center, 2 male and 13 female, median age 63 years, range 16-79; 4 splenectomized and 11 nonsplenectomized, treated with Tpo-RA (Romiplostin n=11 and Eltrombopag n=4). All patients received other ITP drugs as mantenance therapy (eg. costicosteroids, immunoglobulins (IVIG), danazol, azathioprine, rituximab), most of them corticosteroids and IVIG. Romiplostin and Eltrompopag were initiated and monitored according to recommendation described in specification sheet. Hematological response (HR) was defined as PC >=50,000/uL, during 4 or more weeks, without modification of the drugs doses. **Results.** HR was achieved in 11 patients (73. 3%). The median of PC before the treatment was 25,000/uL (4,000-85,000) and post treatment was 90,000/uL (15,000-203,000). The median dose needed to reach HR was Romiplostin 3 ug/kg/week (1-10) vs. Eltrombopag 75 mg/day (50-75). The median time to response was 2 weeks (0-11) and the duration 9 weeks (0-21). Improvement on clinical bleeding was observed in 6 patients (40%) in spite of the absent HR. Concomitant ITP drugs were reduced and discontinued in the 86. 7 % (n=13) and 80 % (n=12) respectively. Rescue medication (corticosteroids and IGIV) was needed in 60 % (n=9) of the patients. Eighty percent of the patients (n=12) did not present any adverse effect related to treatment and the 20% (n=3) showed thrombocytosis and slight haemorrhagic episodes after the treatment discontinuation. PC generally returned to baseline values within 2 weeks after the end of the treatment. None of the patients showed clinical signs or features indicative of bone marrow dysfunction or myelofibrosis. Mortality related to treatment was not observed; a patient died due to pulmonary chronic disease. **Summary and Conclusions.** The news therapeutics (Tpo-RA) are effective and well tolerated treatment for patient with ITP diagnosis. The aim of these therapies is to obtain a safety level that allows diminish the risk of bleeding. In our experience many patients were able to reduce or discontinue other medications and decrease bleeding episodes without few adverse events. This results added to the previous information are similar to the bibliography described up to the date, but it is necessary to increase our experience about that.

1772

A CASE WITH PSEUDOTHROMBOCYTOPENIA WHO SHOWS DIFFERENT FEATURES: TEMPERATURE-INDEPENDENT PSEUDOTHROMBOCYTOPENIA COMPLETE WITH CITRATE AND INCOMPLETE WITH ETHYLENEDIAMINETETRAACETIC ACID AND HEPARIN

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Background. Pseudothrombocytopenia (PTP) is a phenomenon due to agglutination of platelets in the complete blood count tube anticoagulated with ethylenediaminetetraacetic acid (EDTA) leads to falsely low platelet counts. **Aims.** Herein, a case with PTP who shows different features is presented. **Case Report.** A 62 year-old woman referred to our Hematology department from Medical Oncology department because of evaluation of thrombocytopenia. She had colon cancer and colectomy operation was performed two and a half years ago with no need for chemotherapy or radiotherapy. At the time of operation, platelet counts were normal. She had variations in platelet counts during the last year. She has been using lansoprazole for gastroesophageal reflux disease and diosmin/hesperidin for hemorrhoidal complaints for five years. Complete blood count measurements were performed in tubes with citrate and EDTA. Fresh blood samples revealed platelet counts 19 $x10^9$/l in EDTA and 4 $x10^9$/l in citrate tubes. Peripheral blood smear revealed 61% neutrophils, 27% lymphocytes, 9% monocytes, 3% eosinophils with normocytic and normochromic erythrocytes. Platelet clumps were present with mean 9. 3 platelets in every

x1000 magnification. Because of false manipulation of laboratory personnel, the same samples were recounted after three hours platelet values were 50 x10^9/l in EDTA and 10 x10^9/l in citrate tubes, respectively. After these contradictious results, a 24-hour platelet count profile was made by using different anticoagulants (Table 1). According to 24-hour platelet count profile with different anticoagulants, our case had different features from classical EDTA-dependent PTP: PTP was observed in the fresh blood with EDTA, citrate and heparin independently from temperature changes (warm antibodies, not cold agglutinin). In the presence of citrate, antibodies bind platelets more effectively (complete antibody); this effect was incomplete in the presence of EDTA and heparin (incomplete antibody). Therefore, the variations of platelet counts in different hours were observed. Sodium fluoride which is a glycolysis inhibitor explains the false results in tubes with only EDTA and true results in tubes with EDTA combined with sodium fluoride. Sodium fluoride causes inhibition of glucose utilization by platelets in test tube. This leads inhibition of morphological changes of platelets and prevents the formation of PTP. **Conclusions.** As a conclusion, our case which has a temperature-independent PTP complete with citrate and incomplete with EDTA and heparin shows different features from classical EDTA-dependent PTP. True platelet count could be made with addition of sodium fluoride into EDTA.

Table 1. 24-Hour platelet count profile with different anticoagulants.

	0.hour (fresh)	1.hour	2.hour	4.hour	7.hour	24.hour
K_2EDTA 7.2 mg/4.0 ml blood	7*	67	66	51	35	13
Tri-Sodium Citrate 9:1/4.5 ml blood	4	13	20	18	17	17
Lithium Heparin 95 iu/4.0 ml blood	15	22	28	25	34	50
**Sodium Fluoride /K_3EDTA/4.0 ml blood	89	74	69	90	78	20

* Platelet counts were given as x10^9/L.
** This tube contains EDTA and sodium fluoride which causes inhibition of glucose utilization by cells. These tubes are mainly used for glucose and lactate analysis.

1774

IS IT FEASIBLE TO DISCONTINUE TREATMENT WITH ROMIPOOSTIM IN ITP PATIENTS WITH MAINTAINED COMPLETE RESPONSE?

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Background. Primary immune thrombocytopenia (ITP) is an adquired disease characterized by an isolated thrombocytopenia antibody-mediated with destruction and an inadequate production. The main objective of the treatment is to avoid the hemorrhage by keeping the platelets level in a safety range (>20-30x109/L). Although the vast majority of patients respond to steroids (first line), there remains a group of patients who show refractories or steroids dependence, in these cases, it should be considered the use of a second line (splenectomy and thrombopoyetin analogues (a-TPO). The choice of the second line should be an individual decision. The recent incorporation of a-TPO has meant an improvement in the quality of life of these patients by offering them an specific, effective and safety treatment. Its use is limited to adult patients who show refractories to splenectomy or when it is contraindicated or rejected by the patient. **Case Report.** We present two patients with non-splenectomized refractory ITP, who started treatment with Romiplostim reaching manteined complete response (CR) for one year after that we decided discontinue the treatment. 1st: 58 yo male patient with history of ischemic cardiopathy diagnosed of ITP with severe thrombocytopenia <10x10^9/L and cutaneous hemorrhages in July 2008. There was no response after appropriate steroid treatment. Second line treatment is considered, rejecting the splenectomy because of the cardiopathy. 18 months after the diagnosis, refractory disease was stablished, he started treatment with a-TPO. CR with Romiplostim was reached (3ug/kg/week). After 7 months of follow-up and being in CR, it was decided to space out the dose to every 4 weeks. 12 moths after the beginning of Romiplostim, the patient continues in CR. Then, it was decided to definitely discontinue the treatment. Currently, the patient keep a platelet count in the normal range after one year without treatment. 2nd: 19 yo female patient with history of hepatitis B was diagnosed of ITP with metrorrhagia and platelet count <10x10^9/L in July 2010. There was no response after appropiate steroid treatment. The patient rejected the splenectomy. Treatment with a-TPO (Romiplostim) was started 3 months after diagnosis, reaching CR (1. 2 ug/kg/week) After one year being in CR, it was decided to decrease the weekly dose, maintaining the platelet count in the normal range. In December of 2011, the treatment was definitely discontinued. **Conclusions.** Splenectomy is considered the most suitable option as second line treatment with high rate of complete remissions. Besides, exist cases of spontaneous remissions after pharmacologyc treatment in adults with ITP. For this reason, it's advisable to delay the splenectomy at least 6-12 months after diagnosis. A-TPO allow us to achieve CR and delay the splenectomy during this leeway. Although prospective studies are needed, it seems reasonable that patients with maintained CR could finally do without the A-TPO. It does not exist a consensus recommendation related to the withdrawal of the drug. In our cases we used to different alternatives Perhaps it could be considered the progressive weekly decrease of the dose in good responders after one year of treatment, until definitive withdrawal of the drug

1775

ONE YEAR FOLLOW UP STUDY OF ROMIPLOSTIM IN THE TREATMENT OF CHILDREN WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Background. Romiplostim, a thrombopoietic agent with demonstrated efficacy against immune thrombocytopenia is still poorly studied in pediatric age group. **Aims.** Our study aimed to assess the sustained efficacy and safety of romiplostim in the treatment of refractory chronic ITP in children. **Methods.** Ten patients with chronic ITP refractory to standard first and second lines of therapy were recruited from the Pediatric Hematology Unit, Children's Hospital, Ain Shams University, Cairo, Egypt. Therapy was initiated in JUN 2010 through JUL 2011. Romiplostim dose was started as 1µgm/kg/week and the dose was escalated by 1 µg/kg/week according to platelet count. The primary end point was the number of weeks with platelet response, defined as a platelet count ≥50 × 10^9/L. **Results.** Patient age ranged 3. 4 years-15. 2 years(median 5. 5 years),and the disease duration from 13 months-7. 3 years(median 2. 4 years), none was splenectomized. Initial short term rise of platelet count above 100×10^9/L then declined and stabilized at 50-70x10^9/L; regular weekly doses was effective in maintaining platelet count above 50×10^9/L and platelet count rapidly decreased below 20×10^9/L after interrupted therapy. Median dose needed to achieve a platelet count above 50 X10 ^{9}/L was 6ug/kg, and mean duration to achieve this count is five weeks. Most adverse events were mild and transient with no serious adverse events. **Conclusions.** In conclusion, Romiplostim therapy shows variable responses; maintenance doses should be individualized; the drug was well tolerated in studied Egyptian children with ITP .

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RITUXIMAB THERAPY FOR ADULTS WITH CHRONIC AND REFRACTORY PRIMARY IMMUNE THROMBOCYTOPENIA

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Background. In Immune Thrombocytopenic Purpura, platelets are coated with an IgG autoantibody that prompts its premature destruction and, as a result, different grades of peripheral thrombocytopenia become evident. It is important to remember that most patients with ITP do not encounter bleeding problems and it is probably is not necessary to maintain a normal platelet count in all individuals. Treatment is indicated in patients with platelet counts < 30. 000/µl or with bleeding symptoms. First line treatment is traditionally with corticosteroids. In case of failure to steroids, splenectomy induces a 65% response rate. However, almost 30% of adults with ITP fail to respond to standard therapies (steroids, IVIg, anti D) and they develop a chronic refractory disease. If a patient becomes refractory, some alternatives are available such as splenectomy, immunosuppressive drugs, immunomodulatory agents, Rituximab or TPO receptor agonists (second line therapies). **Aims.** Our first objective is to observe the efficacy and safety of Rituximab treatment in adult patients with ITP. A second purpose is to demonstrate the role of Rituximab therapy for chronic and refractory ITP in order to create a "bridge"between standard first line treatment and splenectomy in patients with severe thrombocytopenia and with high bleeding risk. **Methods and Results.** Although the role of Rituximab in treatment of ITP is uncertain, studies have shown that it may work by reduction of antiplatelet antibodies, loss of antigen presentation and resolution of T cell abnormalities (↑ number of Tregs CD4+CD25+Foxp3+). We successfully used Rituximab therapy in case of 2 female patients with chronic and refractory ITP as a "salvage treatment"in order to achieve safe platelet counts before splenectomy. It

was administered as a weekly infusion of 375 mg/m^2 for 3 consecutive weeks and patients did not experienced any adverse effects. In both cases, there was an early pattern of response and their platelet counts were > 75. 000/µl at 28 days after initial dose of Rituximab. During postsplenectomy follow-up period (4-7 months) both patients achieved a complete remission (> 150000/µl). **Conclusions.** Although it is an "off-label"treatment strategy, Rituximab appears to be a safe and good alternative for adults with refractory ITP.

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ELTROMBOPAG IN THE MANAGEMENT OF HEAVILY PRETREATED PATIENT WITH CHRONIC PRIMARY IMMUNE THROMBOCYTOPENIA: A COST-EFFECTIVENESS ANALYSIS IN BRAZIL

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Background. Primary immune thrombocytopenia is characterized by accelerated platelet destruction. Treatment goal includes bleeding prevention by increasing platelet count to stable, safe range. Eltrombopag (Revolade, GlaxoSmithKline), a thrombopoietin (TPO) nonpeptide mimetic that binds to and activates the TPO receptor leading to increased production of megakaryocytes and platelets in bone marrow. **Aims.** The cost-effectiveness of the thrombopoietic receptor agonist eltrombopag versus standard-of-care (SoC) in chronic primary immune thrombocytopenia from an individual heavily pretreated patient viewpoint was assessed. **Methods.** Disease management and model pathways derived from clinical guidelines (PCDT). Health states comprised controlled (>=50,000 platelets/mm3) and uncontrolled ITP (<50,000 platelets/mm3). Time-horizon was continuous, not applied. Cost inputs (drugs, hospital, laboratory, and physician visits) were from public reimbursement databases (BPS/SIGTAP). Eltrombopag price was according to government acquisition requirements (PMVG). Costs were in 2012 Brazilian currency (1BRL=0. 60USD). **Results. Case Report.** The patient is a 62-year-old caucasian woman with a medical history of asymptomatic thrombocytopenia in 2007. After physical examination, and a performance of bone marrow biopsy and aspiration, the patient was diagnosed with primary immune thrombocytopenia. Prednisone was initiated at the dose of 1,5mg/kg/day, with no improvement over a course of 3 months; therefore, immunoglobulin 1g/kg for twice was done, with platelets counts remaining low and never exceeding 20. 000/mm3. Considering the lack of response to corticosteroids and immunoglobulin, immunodepressors were initiated as azathioprine, danazole, and dexametasone in pulses, with no response. Also, rituximab was prescribed for twice. At entirely, the patient received 8 cycles of weekly rituximab over the following months. Because of the heavily treatment, she went to intensive care unit and stayed there for two months, due to a severe pulmonar infection and extended candidiasis esophagitis, that needed thracheostomy. At the time she came to us, she was using prednisone 100mg alternating to 120mg per day, and has just received immunoglobulin 1g/day for two days for nine times in the last two and a half months. Her platelet count was 2,000/mm3. Given the failure of the above therapies, we began eltrombopag, a TPO receptor agonist. After three weeks of treatment with oral eltrombopag at 50 mg daily, the patient's platelet count increased from 2,000 to 6,520/mm3. Six weeks after initiation of eltrombopag, the patient's platelet count increased to 45,200/mm3. In eleven weeks, the platelet count reached 249,000, and the dose was decreased to 25 mg. Nowadays, the patient is well, receiving eltrombopag at 25 mg with no corticosteroids, and with controlled primary immune thrombocytopenia. At the Table 1, the costs for eltrombopag and SoC were calculated and compared. Summary and **Conclusions.** Estimated treatment cost for primary immune thrombocytopenia throughout a 6-month period was BRL21,779 for eltrombopag plus SoC, and BRL153,010 for SoC. Eltrombopag is cost-effective at current international willingness-to-pay (approximately USD50,000/QALY). When higher or repeated immunoglobulin doses are needed for chronic ITP, eltrombopag becomes cost-saving to the Brazilian Ministry of Health.

Table 1.

		Resource use and costs			
		Pre-intervention		Post-intervention	
Treatments	Unit	Total units	Costs	Total units	Costs
Azathyoprine	mg	27000	R$ 231,07	0	R$ -
Danazole	mg	162000	R$ 2.117,02	0	R$ -
Dexametasone	mg	960	R$ 35,95	0	R$ -
Eltrombopag	mg	0	R$ -	5075	R$ 21.297,60
Human immunoglobulin	g	770	R$ 105.644,00	0	R$ -
Prednisone	mg	64050	R$ 105,36	0	R$ -
Rituximab	mg	5100	R$ 44.877,25	0	R$ -
Total cost			R$ 153.010,64		R$ 21.297,60
Weekly cost			R$ 750,05		R$ 1.183,20

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HEMOSTATIC ABNORMALITIES IN CIRRHOSIS AND TUMOR-RELATED PORTAL VEIN THROMBOSIS

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Aims. In order to investigate the relationship between hemostatic abnormalities and portal vein thrombosis (PVT) in hepatocellular carcinoma (HCC). **Methods.** platelets, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time, fibrinogen, D-dimer, fibrinogen degradation products (FDPs), protein C, protein S, antithrombin, plasminogen, antiplasmin, coagulation factors (CFs) V, VII, VIII, IX, XI, and XIII, von Willebrand factor (vWF), prothrombin fragment 1 + 2 (PF 1 + 2), tissue-type plasminogen activator (tPA), and plasminogen activator inhibitor 1 (PAI-1) were studied in patients with HCC, cholangiocarcinoma, and metastatic liver tumors and in cirrhosis patients with or without PVT. **Results.** Platelet, antithrombin, protein C, plasminogen, and CFs V, VII, IX, XI, and XIII levels of HCC group were found lower and PT, aPTT, thrombin time, vWF, FDPs, PF 1 + 2, tPA, and PAI-1 levels were higher than the control group. Summary: Our findings suggested that the abnormalities of coagulation and fibrinolysis systems have some role in provoking thrombosis of portal veins in HCC, in addition to the invasion of portal veins by hepatoma cells.

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HEALTH-RELATED QUALITY OF LIFE RESEARCH IN HEMATOLOGICAL PATIENTS

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Background. EORTC QLQ-C30 as a reliable and valid measure of the quality of life of cancer patients in multicultural clinical research settings. **The purpose.** Our purpose is to determine the differencess between patients with different hematological diseases related to help, pain perception, trouble sleeping, weakness, nausea, appetite, vomiting and fatigue as well as differences in overall quality of life in regard to gender, phase of disease, educational level and employment. **Methods.** A pilot research in a form of a cross sectional study has been conducted at the Department of Clinical Hematology at the Clinical Center of Serbia. The EORTC QLQ-C30 questionnaire has been used. The study includes 50 men (56,2%) and 39 women (43,8%)-average age 52 (21-79)years. 27 (30,3%) patients (pts) with Acute leukemia(AL), 16 (18%) pts with Chronic lymphocytic leukemia (CLL), 12 (13,5%) pts with diffuse large B-cell non-Hodkin lymphoma (NHLDBCL), 8(9 %) pts with non-Hodgkin lymphoma folliculare (NHLF), 9 (10,1%) pts with multiple myeloma (MM) and 9(10,1%) with Hodgkin's disease(HD), 6 (6,7%) pts with thrombocytopenia (ITP) and 2 (2,2%) pts with hemophilia. Data processing was used in the SPSS package. THE **Results.** Most pts had a medium level of education 56(62,9&), high level 14(15,7), primary ili low level 9 (10,1%), higher level 7(7,9%), postgraduate 2(2,2%) and illiterate 1(1,1%). Among them 34(38,2&) were employed, unemployed 29(32,6%) and pensioners 26(32,6%). Phase of disease: initial-40(45%) pts, remission (receving consolidational therapy)- 15(16,8%) pts and relapsed/refractary disease-34 (38,2%). Help with eating, dressing, using the toilet and pain perception was significantly different for patients with MM and hemophilia($p<0,05$). Trouble sleeping was most manifested in AL and CLL patients. Weakness, appetite, nausea, vomiting, fatigue did not differ in groups ($p >0,05$). Tension was most manifested in MM, hemophilia and ITP patients. Overall Health did not differ between the groups, but the overall quality of life is better and significantly differ in NHLDBCL, CLL, hemophilia and HD related to AL, NHLF, MM and ITP. There is also found a gender difference. Male pts had higher quality of life. **Conclusions.** The patients with MM disease have the lowest level of quality of life comparing with other hematological malignacies. The overall quality of life in this group did not depend from phase of disease, educational level and employment.

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AN ESTIMATION OF POTENTIALLY AVOIDABLE AND AMENABLE DEATHS IN SPAIN, 2009 BY RARE HEMATOLOGIC DISEASES

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Background. Rare Diseases or Orphan Diseases (RD) are life-threatening or chronically debilitating diseases with a prevalence lower than 5 cases per 10,000 people, in terms of the prevailing European threshold. Many of the Hematologic Diseases are rare, but while diagnosis for some of them is relatively easy, for others it is delayed, resulting problems with appropriate accurate treatment. Some deaths are considered preventable with timely and effective healthcare: Avoidable and Amenable Deaths, Premature Mortality. These concepts evolve with therapies' improvement. **Aims.** In this work we attempt to estimate the burden of potentially avoidable and amenable deaths inSpain, 2009 from Hematologic Rare Diseases. **Methods.** We use the Anonymous Microdata of Deaths with Cause in Spain, 2009 of the Spanish National Statistics Institute. Rare Hematologic Diseases are selected from Orphanet, after that the equivalence to the International Classification of Diseases (ICD) is searched. To select deaths which would appear to be potentially avoidable we use Hematological Knowledge: the existence of a known effective treatment. In order to choose the amenable deaths we take those accepted in previous scientific literature. **Results.** Despite the limitations of the methods, and the difficult identification of some of the RD in the ICD, this work shows the deaths in Spain, 2009, by Rare Hematologic Diseases, with potentially avoidable and amenable deaths highlighted. There were 1249 deaths as a consequence of Myelodysplastic and Myeloproliferative Diseases. Most of the Myelodysplastic syndromes were diagnosed as "unspecified". More than 230 deaths could be attributed to potentially avoidable deaths, mainly Bleeding Disorders, Anemias, and Hemochromatosis. There were 51 additional deaths secondary to "Human Immunodeficiency Virus disease resulting in other types of Non-Hodgkin Lymphoma". There were 374 cases that could be considered to be due to classically defined potentially amenable deaths (159 by Hodgkin Lymphoma between ages 0 to 74, and 215 by Leukaemia, people 0 to 44 years). According to the incidence data reported in previous literature many of theHematological RD were not even diagnosed. **Conclusions.** In order to prevent potentially significant avoidable premature mortality, there is a need to improve the current knowledge in Rare Hematologic Diseases, evolving in the prophylaxis whenever possible, and later in the identification, characterization, and diagnosis, and moving forward in the sooner and appropriate treatment. The aim of this work is to contribute to foster awareness of Rare Hematologic Diseases.

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CHEMOTHERAPY SAFETY PERFORMANCE: Results FROM A MULTI CENTRIC PROSPECTIVE ANALYSIS

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Background. Risk assessment is crucial for proactively deal with patient safety, and standardised tools are needed to guarantee generalisation and comparability of results. This approach is even more relevant for patients who receive high risk medications, such as chemotherapy. **Aims.** We describe the first Italian experience of setting up and validation of a standardised method for multi centric risk analysis, as part of the Strategic Program: "Towards a complete competence framework and an integrated solution for patient safety in chemotherapy"funded by the Italian Ministry of Health. The Project involved 12 chemotherapy services (Ward andDayHospital) of 9 Italian hospitals. **Methods.** The proposed method starts from the configuration of the present chemotherapy process: the S-Failure Mode Effects Criticality Analysis (S-FMECA) tool shows a complete, codified and validated process mapping of all elementary activities, obtained by means of both literature review and structured interviews to healthcare professionals. In each elementary activity, the analysts identified specific Failure Modes on the basis of a taxonomy of 22 different Error Modes (EM), derived from a review of 48 selected papers about medication errors. All the ways a specific EM may occur are freely described by the analysts for each elementary activity (error dynamics). Rating Scales of Severity, Occurrence, and Detection have been defined to guide the analysts in calculating the Risk Priority Number (RPN) of each activity. The Analytic Hierarchy Process has been adopted to calibrate the four-level Severity Rating Scale through experts' judgments. ADelphimethod has been used to refine the assessment among the participant units. **Results.** The revision of assessments derived from a first application of S-FMECA, considering the risk profiles of the other participants, has allowed the refinement of data. In the subsequent analysis (through the web application FEMC@), several inconsistencies have been deleted. The process consists of seven phases (Patient Admission, Visit, Prescription, Preparation/Dispensing, Distribution, Administration and Patient Monitoring/Discharge). On average, "Preparation"is the phase with the highest RPN (25. 44% of the overall risk), while "Wrong Patient"is the riskiest EM, carrying 17. 10% of the total risk. Clinical centres with similar characteristics have been grouped in clusters and compared each other. No relevant difference in terms of total risk was found comparing computerized and paper-based prescriptions. **Summary and Conclusions.** The proposed S-FMECA method meets the requirements of transparency, reproducibility, and comparability of results. This approach allows an effective benchmark analysis not only of risk values for each phase in the chemotherapy process but also between clusters of similar processes. The results were summarized in reports, distributed to all participants and made public in Workshops, facilitating the comparison with the other Italian services. The web application FMEC@ was particularly appreciated by clinical operators, for whom risk management is typically a complementary subject with respect to their education and practice. On the basis of this results, a continuous monitoring of clinical risk associated to chemotherapy has been implemented by applying statistical quality control charts, according to the ERASMO method.

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IS DEPRESSION A PROBLEM FOR PATIENTS WITH MALIGNANT HEMOPATHIES?

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Background. The diagnosis of malignant hemopathy is a dramatic event in the life of patient in question, with serious repercussions, including common disorders in the individual psyche. The relationship between the psyche and cancer was much discussed. If the mind influence on cancer development is controversial, the psyche is involved at least in quality of life and patient compliance to treatment and thus to its success or failure, with direct consequences for patient survival. **Aims.** We aimed to study the epidemiology and clinical characteristics of patients with hematologic malignancies with and without depression, in order to draw practical Conclusions on their management. **Methods.** We used the Beck's Depression Inventory II to examine 36 consecutive patients, hospitalized in the Hematology Department of Emergency County Clinical Hospital, Sibiu, in February this year, who had a malignant hemopathy diagnosis. We noted gender, age, type of proliferation, the presence of complete remission and diagnosis age (in months). Data were statistically analyzed using the arithmetic mean, standard deviation, Student t test and Pearson test. **Results.** The mean age was 56. 58+/-16. 93 years, the distribution by gender: 21 men (58. 33%) and 15 women (41. 6%). 16. 6% of women and 30. 55% of men were under 60 years. 25% of women and 27. 78% of men were over 60 years. Of the total, 28 (77. 78%) had a diagnosis of AML, MM, NHL, LH and 8 (22. 22%) other diagnoses. Of the 77. 78%, 11 (39. 29%) were in complete remission and 17 (60. 71%) - without complete remission. Mean disease duration was 19. 78+/-23. 43 months. Average score obtained from BDI II was 13. 61+/-8. 72. 5. 57% had severe depression, 16. 6% - moderate, 25% - mild; 47. 23% of patients had a depression score over 13, minimum score for the diagnosis of depression. Only 23% of those with depression were in complete remission, compared with 60% of those without depression. The total duration of disease progression for the 17 patients with depression was 420 months, compared with 292 months - for the others. There was a direct moderate correlation between age and the score obtained (r=0. 44). Regarding gender, women are significantly more often depressed than men (p<0. 05). **Summary.** About half of hospitalized patients suffer from a depressive disorder, most with a mild-moderate form, started during the progression of their hematologic malignancies. Depression is more common in patients with long-term disease, in those who did not obtain complete remission and with age. Women are more commonly affected by depression than men. Elderly and patients with a long and less responsive to treatment malignant hemopathy and especially women will need the attention of clinicians for possible psychotherapy and/or psychiatric treatment, not to jeopardize the results of hematologic therapy.

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END-OF-LIFE, PALLIATIVE CARE AND PLACE OF DEATH: FINDINGS FROM A POPULATION-BASED COHORT OF PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background. This is the first population-based study to address the dearth of empirical evidence about end-of-life, palliative care and place of death in patients with hematological malignancies. Patients with these complex diseases are recognized as being less than half as likely to receive Specialist Palliative Care (SPC) and over twice as likely to die in hospital compared to those with other cancers. The underpinning reasons for this divergence are unknown, although a lack of integration between hematology and SPC has been suggested. **Aims.** To describe the clinical pathways of patients diagnosed with specific hematological malignancies from diagnosis to death, with emphasis on SPC input, place of death and transitions from life prolonging to palliative approaches to care. **Methods.** This study is nested within the UK's Hematological Malignancy Research Network (HMRN - www. hmrn. org): a distinctive collaboration between researchers, a clinical hematology network and a centralized diagnostic laboratory. HMRN routinely collects demographic, treatment, prognostic and outcome data on all patients newly diagnosed with a hematological malignancy (>2,000 per year in a population of around 3. 6 million). The present investigation focuses on acute myeloid leukemia, diffuse large B-cell lymphoma and myeloma in patients > 18 years, that were diagnosed between 2005-2008, and who died before 2010 (n=356). Medical records were abstracted from diagnosis to death using a bespoke day-by-day calendar approach, whereby all hospital episodes were recorded, including information on treating specialities, SPC referrals/input and place of death, as well as decisions around treatment choices, changes in the focus of care, resuscitation status and use of the Liverpool Care Pathway. **Results.** The median age of patients was 74 (range 18-94). Median time from diagnosis to death was 10 months (range 0-54). Approximately one-third of patients were referred for SPC input, which occurred more frequently in patients with longer survival. Hospital was the most common place of death (72%), and in some instances this was the preferred place, whilst only 7% died in a hospice. No evidence was found in the medical records of a discussion about place of death for the majority of patients; however, many of these died soon after diagnosis or were still being treated with curative intent. Individual pathways and the timing of the transition to a palliative approach to care varied greatly by diagnostic group. Further data analysis is on-going and will be presented in the near future. **Conclusions.** The majority of patients with hematological malignancies die in hospital. Many do not receive SPC input; however, it is unclear whether this represents unmet need or whether these needs are met by others - such as haematology clinical nurse specialists. It is our intention to build on this study to promote understanding of end-of-life care in this disease group and we are presently conducting further research to explore reasons why hospital deaths predominate.

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RISK MODELS FOR PREDICTING CHEMOTHERAPY-INDUCED NEUTROPENIA IN PATIENTS WITH SOLID TUMORS AND MALIGNANT LYMPHOMA

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Background. Myelosuppression represents a major toxicity of sistemic cancer chemotherapy associated with considerable morbidity, mortality, and cost. **Aims.** The objective of this study was to develop a model to predicting neutropenia in patients with solid tumours and malignant lymphoma initiating chemotherapy. **Methods.** The study population consisted of 141 patients with solid tumors (56 breast and 40 colon cancer patients) and 45 patients with malignant lymphoma who were beginning a new chemotherapy regimen. Patients had received standard-dose adjuvant chemotherapy for breast (Flourouracil, Adriamycin, Cyclophosphamide) and colon cancer (5 Flourouracil/folinic acid). Patients with lymphoma had received R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin,Vincristine and Prednisone). Neutropenic events reported included neutropenia (defined as an absolute neutrophil count [ANC] 1. 0-1. 5 x 10^9/L [Grade 2] or absolute neutrophil count 0. 5-1. 0 x 10^9/L [Grade 3] or absolute neutrophil count <0. 5 x 10^9/L [Grade 4] with or without body temperature ≥ 38°C). A predictive logistic regression model was developed. Two approaches to modeling were attempted. The pretreatment approach used only pretreatment predictors and the conditional approach included, in addition, blood count information obtained in the first cycle of treatment. **Results.** In the current study, 50 patients (35. 4%) experienced 1 or more neutropenic events during six cycles of therapy. Neutropenic events occurred in a significant proportion of patients (overall 35. 4%, breast 44. 0%, colorectal 10. 0%, lymphoma 46. 0%). The pretreatment model was less successful of predicting neutropenic events (c-statsitic=0. 70) than conditional model. Conditional model was good predictor of subsequent events after cycle 1 (c-statistic=0. 74). Pretreatment absolute neutrophil count (ANC <3. 5 x 10^9/L) was an excellent predictor of events in subsequent cycles (P=0. 002, odds ratio 4. 04) and chemotherapy regiment (P=0. 001, odds ratio 9. 59). The depth of the first-cycle ANC was a significant predictor of events in subsequent cycles (P=0. 009, odds ratio 2. 27). Decline in white cell count during the first cycle of therapy was a significant predictor of neutropenic events (P=0. 030, odds ratio 1. 81). R-CHOP therapy was associated with significant increases in risk over FA/FU therapy (P=0. 000, odds ratio 13. 58) even after accounting for the decline in white cell count during the first cycle and ANC nadir. The sensitivity and specificity of the pretreatment model were 52% and 81% respectively, and the positive and negative predictive values were 54% and 75% respectively. The sensitivity and specificity of the conditional model were 46% and 89% respectively, and the positive and negative predictive values were 70% and 75% respectively. **Conclusions.** It is possible to rank patients according to their need of supportive care based on blood counts observed in the first cycle of therapy. This analysis has implications for patient management and prophylaxis. Additional prospective research is needed to develop more accurate and valid risk models.

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COMPARISON OF SYMPTOM BURDEN OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Hematopoietic stem cell transplantation (HSCT) is an intensive therapy that may cure or control a variety of hematological malignancies. During the first 3 months following HSCT, patients develop symptom burden that interferes with daily activities. Chronic myeloid leukemia (CML) often requires continuous tyrosine kinase inhibitor (TKI) treatment with long-term survival rates of > 80%. TKIs are considered relatively well-tolerated. However, long-term therapy that produces even mild symptoms may produce a substantial burden for patients. **Aims.** The aim of this study was to compare the symptom burden experienced by patients with CML to the symptom burden of patients undergoing HSCT. **Methods.** This is a retrospective analysis of data from a study of symptom burden in 142 patients with CML and a merged data set of 305 patients undergoing HSCT. Both groups responded multiple times to symptom questions on M. D. Anderson Symptom Inventory (MDASI) modules specific for their particular disease and treatment. Symptom burden was measured using the area under the curve (AUC) of the mean of the 5 most severe symptoms for each group. **Results.** The CML patients were slightly older (mean = 51. 8 years; standard deviation [sd] = 13. 4) than the HSCT patients (48. 9, sd =12. 7), more were female (54% versus 43%), more were employed (57% versus 17%), more had better performance status at baseline (ECOG 0-1 = 97% versus 42%), and they had been diagnosed longer (5. 7 years, sd = 4. 8 versus 1. 8, sd = 2. 3). Most CML patients were in chronic phase (97%) on TKI therapy (95%); 42% of the HSCT patients received an allogeneic HSCT. The MDASI assesses symptoms on a scale of 0 (symptom not present) to 10 (symptom as bad as can be imagined). The CML patients responded to 20 symptom items every 2 weeks for 1 year. The HSCT patients from 4 different studies responded to 13 common symptom items at least weekly for the first month following HSCT and then weekly or monthly until 3 months after HSCT. Symptom burden was defined as the AUC of the composite of the 5 most severe symptoms for each group (See Table 1). Because of the difference in the measurement time periods which impacts the AUC, we calculated the average daily mean AUC (CML = 1. 9, HSCT = 2. 3). For the CML patients to reach the AUC that the HSCT patients experienced in 90 days would take 108 days, a difference of only 18 days. **Summary and Conclusions.** TKI therapy for CML has been considered to produce minimal side effects. However, the symptoms experienced by patients with CML on TKI therapy produce burden in approximately 3 ½ months that equates to that of the most intense 3-month period of HSCT. The daily AUC for CML was stable over 1 year, whereas the daily AUC for HSCT was declining at 3 months. Extended over years, this CML burden is substantial. This burden is likely to impact patient functioning and may decrease compliance with therapy. Attention is needed to developing interventions to decrease this unexpectedly high burden.

Table 1. Symptom Burden AUCs for CML patients for 1 year and HSCT patients for 3 months.

CML – 1 year				HSCT – 3 months			
Symptom	N	Mean AUC	Standard Deviation	Symptom	N	Mean AUC	Standard Deviation
Fatigue	142	867.6	704.09	Fatigue	305	257.3	158.11
Muscle soreness	142	670.3	677.10	Physical weakness	305	221.6	159.03
Disturbed sleep	142	666.7	708.50	Lack of appetite	305	200.8	148.34
Drowsiness	142	666.3	631.11	Disturbed sleep	305	189.2	140.09
Trouble remembering	142	551.3	558.87	Pain	305	155.4	134.29
Symptom burden	142	687.3	579.82	Symptom burden	305	205.3	125.60

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PROVIDING APPROPRIATE GENETIC INFORMATION TO HEALTHY CARRIERS OF HEMOGLOBINOPATHY IS A WELCOME AND SAFE INITIATIVE: THE LATIUM EXAMPLE

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Background. Due to massive migration the incidence of hemoglobinopathies is now a day higher in non endemic Northern Europethen in the endemic Southern and Mediterranean countries. Long experience from these countries has shown that diagnose and information of healthy carriers is a welcome and safe initiative. Nevertheless Ethical objections have been raised against informing healthy carriers. **Aims.** To register the opinion and feelings of (presumed) unaware healthy hemoglobinopathy carriers, receiving information on their carrier status. To show to colleagues from non endemic countries involved in primary prevention that properly provided information is a welcome and safe initiative. **Methods.** We have been routinely screening all secondary school children from theLatiumregion (Rome & Central Italy) for several decades, using standard hematological methods. After screening all parents received a standard reassuring letter informing them on the results of the screening and were invited for confirmation of the trait and for additional explanation if needed. During the visit we have collected 259 interviews from parents of students who were provisionally diagnosed hemoglobinopathy carriers. **Results.** We have analyzed 219 interviews (84. 5%) from indigenous subjects and 40 from ethnic minorities (15. 5%). The average age of the parents was 45. 5 years. Only 51 (19. 7%) had previous knowledge of their carrier status while the rest was unaware. When reading the letter with the provisional diagnostic result of their child, emotions that could be considered disagreeable were present in about 60% of the cases. Nevertheless, the information was experienced as welcome, clear and useful by 100% of the participants. Asked about the option of prenatal diagnosis (PD) in case of genetic risk, 85. 7% and 87. 5% of the autochthonous and allochthonous interviewed, declared either to be in favor or eventually to consider PD while only 14. 3% and 12. 5% would not consider it for various reasons. **Discussion.** In spite of ethical objections made in some non endemic countries, we have demonstrated that, if undesirable feelings are present in some of the subjects during the very first reading of the letter, these are only temporary and disappear shortly after or during the visit to the center later on. Significantly, satisfaction and understanding of the advantage of knowledge was registered in 100% of the cases during our enquiry.

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ARE TRANSFUSIONS FOR MYELODYSPLASIA (MDS) AN ACCEPTABLE TREATMENT TO PATIENTS?

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Background. Although the main treatment for MDS is blood transfusions, scarce information is available on patients' experiences of the treatment. Only one relevant study could be identified which reported that patients have a preference for being transfusion independent (Szende et al. Valuation of transfusion-free living in MDS: results of health utility interviews with patients. (2009). *Health Qual Life Outcomes*, 7:81). **Aims**. To investigate MDS patients' experiences with transfusions. **Methods.** Qualitative, unstructured interviews were conducted with MDS patients. Verbatim transcripts were produced and subjected to Interpretative Phenomenological Analysis (IPA). IPA aims at gaining an understanding of patients' experiences from their own perspective. Themes are generated based on issues identified during the interview process. **Results.** The sample included 23 patients with blood transfusion experience (male = 11 (47. 8%); mean (SD) Age=65. 3 (11. 2) mean (SD) time since diagnosis=5. 4 (4. 0)). Of the patients interviewed, 9 (39. 1%) were currently receiving regular transfusions, 5 (21. 7%) had been transfusion dependent previously and 9 (39. 1%) had some transfusion experience. Five major themes were identified in the analyses; positive aspects of transfusions, the cyclic nature of symptoms, transfusion-related limitations on functioning, associated iron chelation therapy and problems with actually receiving the transfusions. Transfusions were perceived positively by some patients as they relieve patients' everyday symptoms. However, these benefits diminish between transfusions leading to cycles of good and poor symptoms, functioning and psychological distress. Limitations to patients' lives resulted from frequent and often long trips to hospital. Negative experiences with the iron chelating drug Deferoxamine were reported by the 3 patients receiving this treatment. The drug is generally administered subcutaneously by means of a battery operated pump and is usually worn for several hours a day causing pain and discomfort. The transfusions were also reported to be boring, uncomfortable and at times painful. **Conclusions.** Transfusions can have a large negative impact on patient's lives. The results suggest there is a need for alternative forms of treatment for patients with MDS. Further quantitative work assessing patients' experiences with transfusions is required.

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DISCLOSURE OF A DIAGNOSIS OF MYELODYSPLASTIC SYNDROME: IMPROVING PATIENTS' UNDERSTANDING AND EXPERIENCE

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Background. How a diagnosis of cancer is disclosed can affect the patient's subsequent psychological morbidity. The specialized terminology of blood malignancies may not be immediately understandable to all patients. **Aims.** We set out to survey how diagnosis of myelodysplastic syndrome (MDS) is announced inFrance and how disclosure is experienced and understood by patients. **Methods.** All 150 members of the French MDS patient support group were sent a questionnaire covering demographic characteristics, circumstances, feelings concerning disclosure, expectations, experiences and comments. In addition, 46 of them were interviewed by a hematologist to collect clinial data concerning their disease. **Results.** Disclosure was experienced negatively by 45% of the 73 patients who returned a useable questionnaire. In retrospect, patients felt they should have been given fuller information at the time of disclosure. They complained it was difficult to understand their disease and the word cancer was mentioned by eight patients. Almost all patients (94%) thought that comprehensive, accurate information should be provided at disclosure, even if the truth might be hard to cope with. However, more than half of the patients also expressed a wish for information tailored to their needs (75%) and for reassurance from the physician (54%), even if that means withholding potentially disturbing information. **Conclusions.** Many patients experience disclosure negatively, frequently finding that the information provided is insufficient and feeling that MDS was not well understood as a disease. Hematologists disclosing MDS diagnosis to patients may benefit from following the same guidelines as oncologists, recommending delivery of comprehensive, understandable information. They clearly recommend using clear, straightforward terminology without avoiding the word cancer when appropriate.

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SALVAGE RADIOTHERAPY, IMPROVES QUALITY OF LIFE LYMPHOMA HODGKIN PATIENTS, AFTER CHEMOTHERAPY FAILURE

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Background. Fox and Lippman have concluded in 1987 y., during follow up 17 patients with advanced Hodgkin lymphoma, that salvage radiotherapy is of significant benefit in patients with advanced Hodgkin's disease relapsing after combination chemotherapy in nodal and/or pulmonary, previously unirradiated sites. Yet, the use of involved-field radiotherapy after chemotherapy for advanced Hodgkin's lymphoma remains controversial. **Aims.** To evaluate efficacy of salvage radiation therapy, in patients with Hodgkin's disease after

chemotherapy failure. **Methods and Materials.** During 2010 and 2011, 5 patients with persistent or recurrent Hodgkin's lymphoma after chemotherapy were treated with salvage radiotherapy. Treatment consisted of extended field radiotherapy to areas of bulky or symptomatic disease. The volume irradiated was mantle field in three patients, inverted-Y in two, and involved-field in one. Total radiation doses ranged from 20 to 40. 0 Gy. Patient characteristics were: age 40-74 years; progressive disease 1/6; stabile disease 2/6; late relapse 2/6; 'B' symptoms 2/6; CS IVB 1/5 and CS IIIB 4/5. Assessments were made using EORTC QLQ-C30(QOL) and the MFI-20(Multidimensional Fatique Inventory questionnaire). Median follow up time was 12 months. **Results.** One patient achieved a complete response. Partial remission was achieved in one patient, MR in two patients and SD in one patient. All patients improved Karnofsky performance status and became asymptomatic . Improvement over time was observed for all scales. Salvage therapy was generally well-tolerated and resulted in no treatment-related deaths. **Conclusions.** Salvage radiotherapy gives significant contribution for improving quality of life and should be considered an option for patients with advanced Hodgkin's disease following chemotherapy failure.

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QUALITY OF LIFE IN PATIENTS WITH HEMATOLOGICAL NEOPLASIC DISSEASES

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The Oncohematologic patient suffers deep somatic and psychological changes while establishing his diagnosis and treatment. In this study the authors have evaluated patient's perception over their own situation and emotional interaction with surrounding sociological factors. A simple and easy testing system including 10 areas of activity, psychodynamic interaction and daily life issues from patients's lifes was elaborated and used by the authors to a group of 65 patients from the Hematology Clinic of Arad and to a control group. A further analysis was to compare the mean values obtained for each of the ten domains of the questionnaire and the items corresponding to each area depending on the variables investigated. Results demonstrated that depending of gender, age, severity of dissease as well as other factors, there is a difference in patient's evaluation. In all items related to health, the patients get results that show lower satisfaction compared with healthy persons. We realise that although in terms of health and professional field the patients have much lower satisfaction compared to healthy people, the disease brings a change of perspective on life and on relationships. Patients are more satisfied than healthy people in certain areas, which provide them anchors they need to deal with the situation in which they live: leisure, relationship with their mate, relationship with their relatives. Family support is particularly important in the fight that oncological patients have to bear with their disease and treatment. As a conclusion, the authors underlined that there can be no promising medical treatment without including social and psychological factors of patients situation during time of hospitalization. Quality of life in oncohematological patients is given by a number of factors, including both health and social relationships, professional and daily life conditions. Perception of quality of life differs in the oncohematological patient to the healthy individual, being perceived differently by gender, stage of disease. The authors also concluded that changes of medical team's view and approach towards the onco-hematological patient are needed. Defining onco-hematological illness as a holistic complex ensemble of somatic and psychological pathological factors this indicates the need for new concepts of treatment in the future.

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IMPLICATION OF POLYMORPHISMS OF BMP6 IN OSTEONECROSIS AMONG SCA PATIENTS IN TUNISIA

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Background. The skeletal manifestations of sickle cell disease are the result of changes in bone and bone marrow caused by the chronic tissue hypoxia that is exacerbated by episodic occlusion of the microcirculation by the abnormal sickle cells. Furthermore, the occurrence of osteonecrosis is under the control of some modifier gene. BMP6 (Bone morphogenetic protein) has been reported as associated with osteonecrosis in sickle cell anemia (SCA). **Aims.** Herein, we entend to study the impact of rs267196, rs267201, rs408505 and rs449853 of BMP6 gene in the occurrence of osteonecrosis among sickle cell patients in Tunisia. **Methods.** Our study involved 100 SCA pediatric patients among whom 50 have osteonecrosis. Rs267196, rs267201, rs408505 and rs449853 of BMP6 gene were analysed for all subjects by PCR/sequencing. We used the software package Arlequin (version 3. 01) for Hardy-Weinberg equilibrium test. Genetic differences according to the presence or not of osteonecrosis were evaluated applying exacts tests to genotypic or allelic contingency tables using compare 2(version 1. 02). **Results.** Our findings show that all samples were found to be in Hardy-Weinberg equilibrium (p>0. 05) for the studied polymorphisms. Furthermore, the distribution of genotypes between SCA patients according to the presence or not of osteonecrosis revealed that patients carried genotype AT of rs 267196 and genotype AG of rs267201 present a high risk factor for developing osteonecrosis OR=5. 66 and OR= 4. 55 respectively. As for rs408505 and rs449853 of BMP6 gene no significant association was found among SCA patients. **Conclusions.** Altogether our data provide that BMP6 can play an important role in developing osteonecrosis.

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A NEW MUTATION OF THE PGK1 GENE DETECTED IN AN ITALIAN PATIENT AFFECTED BY PHOSPHOGLYCERATE KINASE DEFICIENCY

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Background. Phosphoglycerate kinase (PGK) is a key glycolytic enzyme that catalyzes the reversible phosphotransfer reaction from 1,3-bisphosphoglycerate (1,3-BPG) to MgADP to form 3-phosphoglycerate (3-PG) and MgATP. Two isozymes encoded by two distinct genes are present in humans: PGK1 gene is located on Xq-13. 1 and encodes an ubiquitous protein of 417 amino acids, whereas PGK2 is testis-specific. PGK1 deficiency is characterized by mild to severe haemolytic anaemia, neurological dysfunctions and myopathy; patients rarely exhibit all three clinical features. Nearly 40 patients have been reported, 27 of them characterized at DNA or protein level, and 20 different mutations have been described. Recently, we performed biochemical characterization of all PGK1 mutant enzymes to investigate the genotype-phenotype relationship (1). **Aims.** The aim of the study was to characterize at molecular level the first mutation of PGK1 gene found in an Italian patient affected by PGK deficiency. **Methods.** Genomic DNA and total RNA were extracted from leukocytes; the codifying region and intronic flanking regions of PGK1 gene were analyzed by direct sequencing. The PGK1 mutant was obtained from *E. coli* as recombinant protein, purified to homogeneity as previously indicated (1), and assayed for its thermostability and kinetic properties. **Results.** The patient, born from unrelated Italian parents with negative family history, showed at birth neonatal jaundice. During an infective episode at the age of 4, he had haemolytic anaemia (Hb 8. 6 g/dL, reticulocytes 19%, unconjugated bilirubin 0. 91 mg/dL, LDH 445 U/L), increased CPK values (2483-U/L) and respiratory distress. The study of RBC glycolytic enzymes displayed a drastic reduction of PGK activity (41. 8 U/gHb ref. 287-392). The patient was re-examined at the age of25 inoccasion of his sister's first pregnancy. He displayed compensated haemolytic anaemia (Hb 14. 1 g/dL, reticulocytes 6. 6%) and severe myopathy. IQ test indicated mild-moderate mental retardation. PGK1 gene sequencing revealed the new missense mutation c. 1112 T>A (Ile371Lys). The mutation was not found among 100 normal alleles, and even if located in the third last nucleotide of exon 9, it doesn't alter the mRNA splicing. Family study confirmed the X-linked transmission of the disease. Ile371Lys mutation falls in a highly conserved region of the enzyme, near the nucleotide binding site. The mutant enzyme shows reduced catalytic rates toward both substrates (*k*cats, twelve-fold higher than wild-type) and a decreased affinity toward Mg-ATP (apparent *K*m, 6-fold higher than wild-type). Moreover, it lost half of its activity after nearly 9-min incubation at 45°C, a temperature that did not affect the wild type enzyme ($t_{1/2}$ higher than an hour). **Conclusions.** The highly perturbed catalytic properties of the new variant Ile371Lys, combined with protein instability, account for the PGK deficiency found in the patient and correlate with the clinical expression of the disease.

Reference

1. Chiarelli et al, PloS One 2012.

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UNUSUAL HAEMOGLOBIN VARIANTS IN OMAN; PITFALLS IN DIAGNOSIS

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Background. Oman is located on the southeast coast of the Arabian Peninsula. With its unique location, and with its historical connections with the African and Asian continents, many common hemoglobinopathies are present, including the thalassemias, HbS, HbD and HbE. HPLC (high performance liquid chromatography) is a cost effective method of screening for most of the abnormal haemoglobins. Additional tests will increase costs. However, unusual hemoglobinopathies can be encountered and can be mis-diagnosed. **Aims.** To ascertain whether HPLC alone can be used to identify abnormal haemoglobins found in the Omani population. **Methods.** Both complete blood counts (CBC) on a Cell Dyn 4000 automated blood cell counter (Abbott Diagnostics, Santa Clara, CA, USA) and HPLC using the VARIANT IITM instrument (Bio-Rad Laboratories, Hercules, CA, USA) were performed for the blood samples referred for testing for underlying hemoglobinpathy. All newborns born at the hospital were also screened by cord blood HPLC. In addition, all blood samples found to have a mean corpuscular volume (MCV) of less than 70 fL and/or mean cell Hb (MCH) of less than 24 pg undergo analysis. If an abnormality is noted on HPLC analysis (abnormal peaks, abnormal proportion of a variant Hb, incompatibility with hematological parameters), a secondary test is always performed to confirm the diagnostic assumption. This is done through alkaline & acid gel electrophoresis and/or molecular analysis. **Results.** Using the above protocol, 5 unusual hemoglobins variants that needed additional testing were identified. These included:1. Hemoglobin Dhofar [β 29 (GGC-GGT) gly-gly, β 58 (CCT-CGT) pro-arg] is unique to Oman and associated with thalassaemic phenotype. This hemoglobin can be mistaken as Hemoglobin D on HPLC and electrophoresis, but is found at reduced qualities (8. 8%-21. 5% in heterozygotes, 26%-59. 7% in homozygotes). 2. Hemoglobin S Oman: with two β mutations [β6 Glu->Val (HbS mutation) and β121 Glu->Lys (Hb O Arab mutation)]. The heterozygous state results in a mild to moderate sickling syndrome. This hemoglobin can be mistaken for HbC on HPLC and runs close to the C band on alkaline gels. 3. Delta globin variant with Codon 16 mutation can be observed in heterozygotes, homozygotes, and in combination with HbS, HbC, and beta thalassemia. This variant runs in the S window in a very small percentage that can be missed. Underestimation of true A2 level can lead to missing the diagnosis of beta thalassemia trait. 4. Hemoglobin Sheffield [β58(E2)Pro->His] is a phenotypically silent hemoglobin that coelutes in the HbA2 window on HPLC, and can be erroneously diagnosed as HbE. However, it is silent on electrophoresis. 5. β globin promoter -71 C>T mutation. This hemoglobin causes a mild beta+ thalassemic phenotype, increases HbA2 levels in the heterozygous state. When present with an abnormal haemoglobin eg Hb S, the result may be erroneously diagnosed as Hb S trait. **Summary and Conclusions.** If only HPLC is used for diagnosis, pitfalls can occur due to unusual hemoglobin variants. A second confirmatory test should always be done besides HPLC, and in some cases tertiary testing may be necessary.

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ALPHA GLOBIN GENE COPY NUMBER VARIATIONS LEADING TO A FULL ?-THALASSEMIA PHENOTYPE SPECTRUM: A RETROSPECTIVE STUDY

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Introduction. β-thalassemias are caused by a heterogeneous group of genetic alterations characterized by a deficient synthesis (β+) or an absence (β0) of β globin chains in that locus. Thalassemic phenotypes include asymptomatic cases (thalassemia minor; in most heterozygote forms of β-thalassemia) and patients suffering a transfusion dependent severe form of the disease (thalassemia major; in homizygote or double heterozygote patients). β globin chain production in the red cells is the main phenotype modifier in β-thalassemia. Nevertheless, the are other modifiers that can ameliorate the severity of the phenotype (elevated HbF levels) or enhance it (increased α/β ratio caused by extra α globin gene copies). **Aims.** This is a descriptive study showing a cohort of β-thalassemia patients (β+ or β0) carrying heterozygous or homozygous αanti 3. 7Kb duplications (ααα/αα or ααα/ααα conditions, respectively). **Methods.** Hematometric data were obtained in a GENS Coulter. Quantification of HbA2 and HbF levels was done by HPLC (Variant II). Screening for β gene mutations was performed by sequencing and α globin copy number was measured by Multiplex Ligation-dependent Probe Amplification (MLPA) technique. Patients were divided in four groups according to their genotype: Group1 (β0 plus ααα/ααα); group2 (β0 or severe β+ plus ααα/αα); group3 (mild β+ plus ααα/ααα) and group4 (mild β+ plus ααα/αα). A total of 38 patients were included in this study. **Results.**The following β gene mutations have been included here: βCD39 (β0 condition, 15 cases); βIVS-I nt-1 (β0 condition, 12 cases); βCD82/83 (-G) (β0 condition, 2 cases); βIC (ATG >) (severe β+ condition, 1 case); βIVS-II nt-654 (severe β+ condition, 1 case) βIVS-I nt-110 (severe β+ condition, 5 cases) and βIVS-I nt-6 (mild β+ condition, 2 cases). Results are summarized in figure 1. DISCUSSION. Here we show a cohort of patients with all the possible combinations between both β+ or β0 gene mutations and the αanti 3. 7Kb duplication. The phenotype manifestations widely differs between patients, presenting severe β-thalassemia (major or occasionally transfusion thalassemia intermedia), mild thalassemia intermedia and β-thalassemia trait. Severe thalassemia is observed in patients with the greater α/β imbalance. These cases correspond to β0 mutations associated with ααα/αααα, in which β chain production is severely compromised (patients I and II-I). Mild thalassemia intermedia phenotype is observed when a β0 or severe β+ mutation is present in association with αanti 3. 7Kb duplication in heterozygous state (ααα/αα), thus, α/β imbalance is not as prominent as seen in the patients mentioned above. Interestingly, this phenotype is seen in patient with the mild IVS-Int-6 β+ mutation associated to ααα/αααα. In this particular case α/β imbalance is caused mainly by the α globin over expression. Thalassemia trait is shown by patient XXVI-II (mild β+ mutation plus ααα/αααα) and one patient in group2 (β0 mutation plus -α3. 7/αααanti 3. 7, with no net α copy number variation). In both patients α/β ratio should be similar but reached with different α and β chain contributions in each case. The different phenotypes exposed here manifest how important issue is the α/non-α globin chain production in order to modulate the β-thalassemia clinical expression.

Table 1. Results summary.

Group	Patient	Age/ Sex	Hb (g/dL)	MVC (fL)	MCH (pg)	RDW (%)	Ret (%)	HbA2 (%)	HbF (%)	α-gen	β-Mutation	Phenotipe
I (β+/ ααα/ααα)	I	14/M	8.3*§	57.5	18.7	17.7	4.4	5.0	2.0	ααα/ααα	CD39	Severe Thalassemia Intermedia
	II-1	45/M	6.3*§	88.8	25.9	29.2	2.3	2.8	27	ααα/ααα	CD82/83 (-G)	Severe Thalassemia Intermedia
II (β+/ ααα/αα or severe β+/ ααα/ααα)	III	29/F	9.8	60.6	20.5	11.7	1.2	3.6	1.0	ααα/αα	CD39	Mild Thalassemia Intermedia
	IV	2/F	8.3	56.2	18.0	19.0	3.0	4.0	5.5	ααα/αα	CD39	Mild Thalassemia Intermedia
	V-I	8/M	10.5	65.0	20.0	19.0	0.6	5.6	1.3	ααα/αα	CD39	Mild Thalassemia Intermedia
	V-II	3/M	10.6	58.0	19.0	19.8	0.5	5.2	2.1	ααα/αα	CD39	Mild Thalassemia Intermedia
	VI	32/M	9.1	61.2	19.6	17.1	2.1	4.3	3.5	ααα/αα	CD39	Mild Thalassemia Intermedia
	VII	30/F	8.3	74.0	22.6	21.2	5.1	4.8	1.2	ααα/αα	CD39	Mild Thalassemia Intermedia
	VII	59/M	10.4§	65.4	20.8	17.3	1.3	4.0	2.8	ααα/αα	CD39	Mild Thalassemia Intermedia
	IX	32/M	9.3	72.0	21.5	21.1	3.0	4.6	10	ααα/αα	CD39	Mild Thalassemia Intermedia
	X-I	16/M	8.6	57.8	18.3	21.6	3.0	5.8	3.0	ααα/αα	CD39	Mild Thalassemia Intermedia
	X-II	7/F	8.1	60.3	19.8	24.6	3.2	5.2	5.9	ααα/αα	CD39	Mild Thalassemia Intermedia
	XI-I	50/M	10.7	60.2	19.2	17.4	0.8	4.8	0.5	ααα/αα	CD39	Mild Thalassemia Intermedia
	XI-II	23/F	9.7	57.9	18.8	21.5	2.0	4.8	5.7	ααα/αα	CD39	Mild Thalassemia Intermedia
	XII	58/M	9.3	81.3	25.3	26.8	10.4	4.0	10.0	ααα/αα	CD39	Mild Thalassemia Intermedia
	XIII	42/M	10.5	70.4	20.8	23.1	3.7	5.5	3.1	ααα/αα	CD39	Mild Thalassemia Intermedia
	II-II	12/F	8.8	62.8	19.4	18.4	2.33	5.6	1.1	ααα/αα	CD82/83 (-G)	Mild Thalassemia Intermedia
	XIV	35/F	9.2	66.5	21.72	33.1	4.4	4.0	4.5	ααα/αα	IC (Atg>Gtg)	Mild Thalassemia Intermedia
	XV	20/F	9.9	62.1	20.8	14.3	1.1	5.0	3.0	ααα/αα	IVS-I nt-1	Mild Thalassemia Intermedia
	XVI-I	25/F	11.0	60.0	20.5	15.7	1.1	3.8	1.8	ααα/αα	IVS-I nt-1	Mild Thalassemia Intermedia
	XVI-II	6m/F	9.4	61.0	20.0	18.0	1.2	3.6	8.0	ααα/αα	IVS-I nt-1	Mild Thalassemia Intermedia
	XVII	60/F	9.1	74.5	26.0	26.0	5.5	5.0	4.5	ααα/αα	IVS-I nt-1	Mild Thalassemia Intermedia
	XVIII-I	26/M	12.5	66.1	21.1	15.4	1.0	5.0	3.0	ααα/-α3.7	IVS-I nt-1	Thalassemia trait
	XVIII-II	18/M	10.4	65.4	20.8	17.3	1.3	4.0	2.8	ααα/ααα	IVS-I nt-1	Mild Thalassemia Intermedia
	XIX-I	23/F	9.6	63.9	21.1	16.5	1.6	5.0	6.0	ααα/ααα	IVS-I nt-1	Mild Thalassemia Intermedia
	XIX-II	10/F	10.6	57.3	18.7	16.8	0.9	5.0	8.0	ααα/ααα	IVS-I nt-1	Mild Thalassemia Intermedia
	XX-I	20/F	7.2**	72.4	20.1	22.5	3.7	4.8	4.0	ααα/ααα	IVS-I nt-1	Mild Thalassemia Intermedia
	XX-II	24/M	10.4	60.7	18.4	17.3	1.9	5.6	6.0	ααα/ααα	IVS-I nt-1	Mild Thalassemia Intermedia
	XXI-I	23/M	10.4	60.7	18.4	17.3	1.9	5.0	4.0	ααα/ααα	IVS-I nt-1	Mild Thalassemia Intermedia
	XXI-II	25/F	9.2	64.4	19.1	16.5	2.1	4.8	3.9	ααα/ααα	IVS-I nt-1	Mild Thalassemia Intermedia
	XXII-I	38/M	11.0	59.3	18.5	16.5	3.0	3.7	2.0	ααα/αα	IVS-I nt-110	Mild Thalassemia Intermedia
	XXII-II	10/M	10.8	60.3	18.7	16.6	2.0	4.4	1.2	ααα/αα	IVS-I nt-110	Mild Thalassemia Intermedia
	XXIII-I	22/F	8.2**	63.4	20.6	16.1	2.4	4.3	3.5	ααα/αα	IVS-I nt-110	Mild Thalassemia Intermedia
	XXIII-II	20/F	10.3	61.2	19.6	17.1	2.1	4.2	3.1	ααα/αα	IVS-I nt-110	Mild Thalassemia Intermedia
	XXIV	21/F	8.6	63.4	20.6	16.1	2.4	4.0	3.0	ααα/αα	IVS-I nt-110	Mild Thalassemia Intermedia
	XXV	11/M	10.1	60.2	18.4	16.6	2.2	6.6	0.9	ααα/αα	IVS-II nt-654	Mild Thalassemia Intermedia
III (mild β+/ ααα/ααα)	XXVI-I	33/F	9.8	73.1	23.2	16.3	3.8	3.9	1	ααα/ααα	IVS-I nt-6	Mild Thalassemia Intermedia
IV (mild β+/ ααα/αα)	XXVI-II	60/M	14.5	74.5	23.5	16.2	1.94	3.8	0.9	ααα/αα	IVS-I nt-6	Thalassemia trait

Table 1: β-thalassemia associated with α-gene triplications Where: *: transfusion dependent. **: pregnant. §: deferroxamine chelation

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A CASE OF CONGENITAL RED CELL PYRUVATE KINASE DEFICIENCY ASSOCIATED WITH HEREDITARY SPHEROCYTOSIS

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Pyruvate kinase (PK) deficiency, transmitted as an autosomal recessive trait, is the most common erythroenzymopathy of glycolytic pathway (prevalence of 1:20,000) associated with chronic non spherocytic hemolytic anemia from mild to severe. More than 180 mutations in the *PK-LR* gene have been so far reported, and genotype-phenotype correlation has been established for some of them. Hereditary Spherocytosis (HS) is the most common congenital hemolytic anemia in Caucasians, with an estimated prevalence ranging from 1:2000 to 1:5000. The main clinical features are hemolytic anemia from compensated to severe, variable jaundice, splenomegaly and cholelythiasis. The molecular defect is highly heterogeneous, caused by proteins involved in the attachment of cytoskeleton to the membrane integral domain (spectrin, ankyrin, band 3 and protein 4. 2). We describe a case of PK deficiency associated with HS. The propositus was a 13 years-old Italian male with neonatal jaundice and need of blood transfusion (Hb 5. 8 g/dL) during an infectious episode. At the time of the study Hb was 13. 9 g/dL, MCV 81. 8 fL, reticulocytes 207x10^9/L, unconjugated bilirubin 2. 16 mg/dL, LDH 605 U/L, haptoglobin <20 mg/dL. The peripheral blood smear examination showed the presence of spherocytes (16%) and some ovalocytes (2%). The study of the most important red cell enzymes

revealed reduced PK activity (59% of normal). Direct sequencing of *PK-LR* gene showed compound heterozygosity for the 994A mutation (Gly332Ser) and the -148T variant localized the erythroid specific promoter region. The presence of spherocytes in peripheral blood smear prompted us to investigate for the coexistence of HS. Erythrocyte osmotic fragility was decreased and SDS-PAGE analysis of red cell membrane proteins revealed a 30% spectrin reduction. Family study demonstrated a heterozygous condition for the 994A mutation in the father, who also displayed comparable enzyme deficiency, whereas promoter variant -148T was detected in the mother and in the brother. No red cell membrane abnormalities were present in the family members, although positive EMA binding test and increased osmotic fragility were found in the father and brother. The co-existence of HS and PK deficiency is very rare event, only few cases are described to date. Clinical, family and molecular studies allowed the determination of the interrelationship between the two RBC abnormalities in the patient and his relatives. The reduced PK activity in the propositus and his father is justified by heterozygous 994A mutation. The more severe clinical picture in the propositus could be caused by the coexistence of HS and by the presence of -148T mutation, that although it seems not to have effects on *PK-LR* mRNA expression, is often detected in PK deficient subjects with heterozygous PK mutations.

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HOMOZYGOUS AND HETEROZYGOUS HEMOGLOBIN S ASSOCIATED WITH HEMOGLOBIN G-PHILADELPHIA

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Background. Electrophoresis of hemoglobins (Hb) and HPLC techniques are usually sufficient for the identification of HbS. However, the association with an α-globin chain structural variant produce results difficult to interpretate with these methods. This is caused by the formation of a new Hb that contains both the two abnormal β and α-globin chain variants. **Aims.** In this paper we present the electrophoretic, chromatographic and molecular association of an heterozygous HbS and another homozygous HbS with the α-chain variant G-Philadelphia. **Methods.** Case 1: 49-years-old man referred with a sickle cell anemia diagnosis. Case 2: asymptomatic 31-years-old woman. The Hbs' study was made by electrophoresis at alkaline pH (8. 6), and acid pH (6. 0), capillary electrophoresis, ion exchange HPLC and reverse phase HPLC. We studied the existence of an α-thalassemia by gap-PCR. Molecular analysis was completed by automatic sequencing of PCR amplification products of genes α_1, α_2 and β. **Results.** In case 1, by alkaline pH electrophoresis were observed three Hbs, one at the level of HbA (corresponding to $\alpha^A\beta^A$ transfused Hb), another at the level of HbS, G, D (corresponding to $\alpha^A\beta^S$ Hb) and the last one at the level of HbC, E, O-Arab (corresponding to $\alpha^G\beta^S$ Hb). Only two bands were observed at acid pH, one at the level of HbA, E, D (corresponding to $\alpha^A\beta^A$ transfused Hb) and another at the level of HbS, O-Arab (corresponding to $\alpha^G\beta^A$ and $\alpha^G\beta^S$ Hbs). By ion exchange HPLC three elution peaks corresponding to αAβA Hbs (transfused), $\alpha^G\beta^A$ and $\alpha^G\beta^S$ were showed. By reverse phase HPLC 4 peaks corresponding to β^S, β^A, α^G and α^A chains were observed. In case 2, by ion exchange HPLC were observed 4 elution peaks corresponding to $\alpha^A\beta^A$, $\alpha^A\beta^S$, $\alpha^G\beta^A$ and $\alpha^G\beta^S$. By reverse phase HPLC were observed 4 peaks corresponding to β^S, β^A, α^G and α^A β^S chains. Both patients had a loss of a functional alpha gene by the 3. 7 Kb deletion in one allele. The sequencing of the gene in the molecular study of first case showed GAG → GTG mutation at codon 6 of β gene in the homozygous state and in the second case in the heterozygous state (β6 (A3) Glu>Val). In sequencing of the α genes was demonstrated the AAC → AAG mutation at codon 68 of the $\alpha_2\alpha_1$ hybrid gene formed by the deletion of 3. 7 kb in both cases (α68 (E17) Asn> Lys). **Conclusions.** The sickle cell anemia may be due to the combination of HbS with others Hbs or thalassemic syndromes. This event make the diagnosis more difficult. In both cases, the integration of electrophoretic, chromatographic and molecular studys allowed for a correct diagnosis.

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CHARACTERIZATION OF 3 HEMOGLOBINOPATHIES THAT PRESENT CYANOSIS AND DESCENT OF THE SATURATION OF 02

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Background. Structural hemoglobinopathies along with thalassemia are the most common monogenic disorders worldwide. Depending on the nature and location of the substituted aminoacid are to determine changes in the stability, solubility and function of the hemoglobin molecule that are ultimately responsible for the clinical manifestations of hemoglobinopathies. **Aims.** To present the molecular characterization of three hemoglobins variants that his suspicion was conducted by the existence of a decrease in oxygen saturation with cyanosis. **Methods.** We studied 3 cases (1: M/35 years old, 2: M/34 years old, 3: F/19 years old) referred to have decreased oxygen saturation and cyanosis. In all 3 cases, by ion exchange HPLC abnormal hemoglobin was eluted behind the HbA. By reverse phase HPLC was separated abnormal α chain in the first 2 cases and an abnormal β chain in the third. **Results.** In sequencing of the globin genes was observed:Case 1: CAC>TAC mutation at codon 58 of α_2 gene in heterozygous state that determines the change of histidine by tyrosine, known as Hb M-Boston (α_2 58 (E7) His→Tyr). Case 2: CAC>TAC mutation at codon 94 of α_2 gene in heterozygous state that determines the change of aspartic by asparagine, known as Hb Titusville (α_2 94 (G1) Asp→Asn). Case 3: CAT>TAT mutation at codon 63 of the β gene in heterozygous state that determines the change of histidine by tyrosine, known as Hb M-Saskatoon (β63 (E7) His→Tyr). **Conclusions.** In the Hb M- Boston and M-Saskatoon changing the proximal histidine for tyrosine at heme group at the anchor of α and β chains respectively. This fact, determines that the heme central iron was oxidized permanently to yield up an electron and therefore it can not capture oxygen, this is the cause of the decrease in oxygen saturation and cyanosis. In Hb Titusville aspartic changing by asparagine at position G1 of α chain, that it was involved in the $\alpha_1\beta_2$ contact and determines the stabilization of the Hb in conformation T or deoxy therefore has a low affinity for oxygen being the reason of decreased oxygen saturation and cyanosis. The possibility of the presence of one structural hemoglobinopathies must be taken into account in cases of cyanosis and decreased oxygen saturation without apparent cause. Although not present a significant clinical impact, their confirmation saves people carrying large and unnecessary studies.

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BCL-2 LEVELS IN PATIENTS WITH THALASSEMIA MINOR

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Background. Antiapoptotic proteins such as bcl-2 and bcl XL may play a role on the long-life of erythroid progenitor cells. Data about these proteins in patients with thalassemia minor is limited. **Aims.** We aimed to investigate the levels of bcl-2 in patients with thalassemia minor. **Methods.** Ninety-seven patients (60 female and 37 male, with mean age of 29 ±21 years) with thalassemia minor were enrolled to this study. Thalassemia minor was diagnosed with complete blood count, family history, and HbA2 levels using by HPLC. 23 healthy persons (17 female and 6 male, with mean age of 58 ± 9 years) were received as a control group. Whole blood cell counts were analyzed with Beckman-Coulter instrument. The levels of bcl-2 were measured by using commercial ELISA kit (Biosource Cat No: TMA 0311, Camarillo, California, USA). Mann-Whitney U test were used for statistically comparison of results and p< 0. 05 was accepted as statistically significant. **Results.** There was no statistically significant between patients with thalassemia minor and control group for the level of bcl-2 (p>0. 05). Mean corpuscular volume (MCV), the levels of hemoglobin and bcl-2 were given (Table 1). **Summary and Conclusions.** Beta chains are damaged in thalassemia minor. Therefore, it is expected that premature death of red blood cells are related to apoptosis. The level of bcl-2 (antiapoptotic protein) in was statistically non-significant higher in thalassemia minor than control. The mean age of control group was higher than thalassemia minor and it is known that older age is risk for more apoptosis. The evaluation of other proteins (bad, bax, etc.), pathways (CD 95 fas ligand) associated with apoptosis and higher number of patients will contribute to determine about this topic.

Table 1. MCV, the levels of hemoglobin and bcl-2 in patients with thalassemia minor and control group.

	Thalassemia minor (n: 97)	Control (n: 23)	p-value
hemoglobin (g/dl)	11,4±1,3	13,7 ±1,1	<0.001
MCV (fl)	64±8	86 ±3,4	<0.001
level of serum bcl-2 (ng/ml)	34.2±7.6	32.7±13	>0.05

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HEMOCHROMATOSIS AND PROTHROMBOTIC MUTATIONS CO-INHERITANCE COULD REPRESENT A RELATIVE SELECTION ADVANTAGE DURING PREGNANCY AND CHILD BIRTH

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Background. Iron deficiency is a frequent situation in premenopausal women and even more so in pregnancy. One can postulate that having a hemochromatosis gene, present in up to 10% in the Caucasian population, could therefore represent a biological advantage. In the Caucasian population prothrombotic mutations are also present with an allele frequency of about 4 to 7% for V Leiden (VL) and 1 to 3 % for prothrombin (PT). These mutations account for an increased risk of thromboembolic events and this risk is considerably magnified during pregnancy. **Aims and Methods.** One could reasonably expect that the disadvantage of a thrombophilic mutations is offset by some other selection advantage, especially in the pregnant state, explaining their present day prevalence. Because of this hypothesis we studied the genotype frequencies of hemochromatosis (C282Y and H63D) and the prothrombotic state (PT, VL and MTHFR) in samples we had in our DNA bank from random healthy volunteers. We included MTHFR, though it is rather a polymorphism, present in about 40% of the Caucasian population in our area, and its prothrombotic effect is not significant when not associated to other risk factors. **Results.** Table 1. H63D and VLeiden alleles correlate significantly, p= 0. 0448. **Conclusions.** We suggest that the association of increased iron absorption and a decrease of bleeding risk during childbirth could have resulted in a selection advantage for each entity and even more so for a combination of both. From a Darwinian point of view the heterozygosity association of VL and H63D is remarkable, as the prevalence of VL is higher than PT and that H63D increases moderately iron absorption but is a negligible risk factor for deleterious clinical hemochromatosis.

Table 1. Pair-wise tests of association between the two sets of genotype frequencies.

N of individuals	119	151	124
	PT	VL	MTHFR
C282Y	p= 0.0034	p= 0.959	p=0.872
H63A	p= 0.782	p= 0.0448	p= 0.053

1800

SNPS POLYMORPHISM OF ?-GLOBIN CLUSTER AMONG TUNISIAN SICKLE CELL PATIENTS: IMPLICATIONS FOR CLINICAL DIVERSITY

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Background. The sickle cell disease (SCD) is the most common single gene disorder in the world. In spite of the discovery of the molecular basis of SCD, the causes of the phenotypic heterogeneity of the disease remain unclear. This heterogeneity cannot be explained by the single mutation in the beta-globin gene alone but may be attributed to genetic single nucleotide polymorphisms (SNPs) in the β-globin gene cluster. **Aims.** We evaluated the effects of polymorphic markers within the β-globin gene cluster on HbF expression and clinical diversity in Tunisian patients. **Methods.** Haplotype analysis was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the framework polymorphism was established by direct sequencing, we examined the locus control region hypersensitive sites two (LCR-HS2) of the β-globin gene (AT)xN(AT)y, an IVSII to γ^A and γ^G gene $(TG)_n(CG)_m$ and the region of 5' to β-globin gene (AT)xTy repeat motifs. Correlation of these various haplotypes and SNPs with HbF expression and clinical data was studied. **Results.** Of the various polymorphic markers analysed, only the LCR HS2 (AT)xN(AT)y sequence configurations were significantly associated with increased HbF levels, and the 5' region of β-globine gene (AT)xTy were significantly associated with disease severity, suggesting that the β-globin gene cluster exerts a significant effect on HbF and clinical expression in sickle cell patients. **Summary and Conclusions.** The discovery of SNPS implicated in different phenotypes will help understanding of the physiopathology of the disease and aid to increase our ability to predict clinical severity.

1801

GHRELIN GENE POLYMORPHISM IN VITAMIN B12 DEFICIENCY

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Background. Vitamin B12 is seen in 3-66% of the cases. In our country, it is detected in 5. 9% of the adolescents in the 7-17 age group. Ghrelin stimulates appetite, and food intake. Appetite is regulated by cerebral center. Ghrelin containing neurons were detected in the arcuate nucleus of hypothalamus. One of the clinical features of vitamin B12 deficiency is loss of appetite. In iron deficiency anemia which is also a nutritional anemia, loss of appetite was detected although at a lesser extent. Decrease in the ghrelin level or some polymorphisms (promoter -501 A/C, Arg51Gln, Leu72Met, Gln90Leu) developing in its gene might be the common etiologic factor for both types of nutritional anemia. **Aims.** In vitamin B12 deficiency, levels of ghrelin, and the stated 4 polymorphisms were analyzed to clarify whether or not these factors might be the cause of this disease state. **Methods**. Forty-two cases with vitamin B12 deficiency, and equal number of healthy controls were evaluated. Informed consent was obtained. Ghrelin (achyl ve deachyl ghrelin) levels, and promoter -501 A/C, Arg51Gln, Leu72Met ve Gln90Leu polymorphisms in its gene in patients, and control subjects were investigated. Independent Samples t test was used, and p<0. 05 values were considered significant. **Results.** In patients, and controls, levels of achyl (14. 510. 0 pg/ml, and 24. 214. 2 pg/ml, respectively) and deachyl ghrelin (242. 3206 pg/ml, and 394. 0170 pg/ml, respectively)were determined (p<0. 05). Statisticaly -501 A/C polymorphism in ghrelin gene as CC genotype is found increased frequency in patient group (p<0. 05). For Gln90Leu polymorphism in group heterozygot Gln/Leu genotype is found in increased frequency (p<0. 05). **Summary.** Lower levels of ghrelin are observed in vitamin B12 deficiency. Further studies are needed to determine the potential contribution of gene polymorphisms to the development of vitamin B12 deficiency.

1802

EFFECTS OF IRON DEFICIENCY ANEMIA AND ITS TREATMENT ON GHRELINS, OBESTATIN AND HEAT SHOCK PROTEIN 70

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Background. In the human body there exists a positive correlation between iron stores, and ghrelin levels. Under stressful conditions, mostly heat shock protein 70 (HSP70) is affected. Release of HSP 70 is stimulated by ghrelin. The exact levels of HSP70 in iron deficiency anemia (IDA) are unknown. **Aims.** The impact of IDA, and its oral treatment on increased levels of (HSP70 in settings with higher tissue stress induced by both ghrelin which is both an antioxidant, and a food intake stimulant, and also obestatin with opposing effects was investigated. The association of pica with these parameters was also examined. **Methods**. The study population included 28 patients with IDA, and 28 healthy controls. **Results.** While acyl ve desacyl ghrelin values were lower (p<0. 05) in IDA than those of the control subjects, with treatment ghrelin levels in both groups climbed to relatively higher levels (p>0. 05) than the control group. In IDA obestatin levels were higher than the control values (p<0. 05) which still persisted at elevated levels during the treatment period (p>0. 05) With treatment of IDA, the levels of acyl, and desacyl ghrelin increased, contrarily obestatin values fell down. Also, the concentration of HSP 70 in IDA, and during its therapy (peak value) was above control values (p>0. 05). Acyl ghrelin, desacyl ghrelin, and HSP70 levels were increased in the pica group, while obestatin was found to be decreased (p>0. 05). In the pica group obestatin/acyl ghrelin, and obestatin/desacyl ghrelin ratios were comparatively higher (p<0. 05). **Summary and Conclusions. .** In IDA decrease in ghrelin, and increase in obestatin levels are observed, while HSP 70 remains the same. Increases in obestatin/acyl ghrelin, and obestatin/desacyl ghrelin ratios might be responsible for the pica disorder.

1803

ASSOCIATION OF NT +20 (C>T) AND IVS-II-745 (C>G) β GENE MUTATIONS IN CIS

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Background. The 5 'UTR of the β gene is an area that is transcribed but not translated. 5 'UTR is involved in post-transcriptional gene regulating, favoring and stabilizing the mRNA translation. To date, have been described mutations in the nucleotide +22 (G>A) and +33 (C> G) by different mechanisms mild decrease of β gene expression leading to β+ phenotype or silent. Also described a mutation at nucleotide +20 (C>T) in the database 'Human Hemoglobin Variants and thalassemias' (http://globin. bx. psu. edu/hbvar/menu. html) to cause β^+ thalassemia resulting in a phenotype of thalassemia minor in heterozygous cases and thalassemia intermedia associated with β^+ IVS-II-745 (C>G) mutation. **Aims.** We report our experience in eight cases in which we have found mutations nt +20 (C>T) and IVS-II-745 (C>G). MethodsWe have study five families, a total 10 patients. Three thalassemia major transfused dependent and the rest (7) thalassaemia trait. Molecular characteization was made by automatic sequencing of β gene globin and the α-thalassemia by stripAssay. **Results.** Table 1. **Conclusions.** The presence of both mutations nt +20 (C>T) and IVS-II-745 (C>G) in homozygous state in one patient (III_1) with thalassemia major suggests that these mutations were found in cis in the same allele. This finding had been corroborated by another cases of thalassemia major (I_2 and IV_3) in which both mutations nt +20 (C>T) and IVS-II-745 (C>G) were inherited in cis from one of the parents ($I_1$and IV_1) and the other mutation [CD8(-AA) and CD39(C>T)] was presented in the other parents (I_3 and IV_2). The phenotypic expression of the other cases (II and V), which showed only mutations nt +20 (C>T) and IVS-II-745 (C>G), were of a minor thalassemia as in I_1 and IV_1, suggesting that also in these cases are in cis in the same allele. These results suggest, contrary to reports in the database "Human Hemoglobin Variants and thalassemias', that the mutation nt +20 (C>T) would be a polymorphism that is associated with the mutation and IVS- II-745 (C>G) in cis and not likely to influence gene expression.

Table 1. Summary of hematogical parameters, genotype and phenotype of the patients.

Patients	Sex/Age	Hb (g/dL)	MCV (fL)	HbA2 (%)	HbF (%)	Genotype	Phenotype
I1	M/41	13.5	60.6	4.8	1.1	+20/2-745 αα/αα	Th. Minor
I2	M/2	7.6	81	2.8	25	+20/2-745 CD8 αα/αα	Th. Major*
I3	F/21	11.5	63.6	4.9	0.5	CD8 αα/αα	Th. Minor
II	M/31	14	62.7	3.9	1.6	+20/2-745 αα/αα	Th. Minor
III1	F/25	13.5	60.6	4.8	1.1	+20/2-745 +20/2-745 αα/αα	Th. Major*
III2	F/48	10.0	67.3	5.2	1.4	+20/2-745 αα/αα	Th. Minor
IV1	M/27	13.2	69.0	5.2	0.5	+20/2-745 $-\alpha^{3.7}/\alpha\alpha$	Th. Minor
IV2	F/24	10.5	70.8	4.9	3.1	CD39 αα/αα	Th. Minor
IV3	M/4	9.9	88.6	2.5	0.6	+20/2-745 CD39 αα/αα	Th. Major*
V	M/34	12.8	64.9	4.7	1.1	+20/2-745 αα/αα	Th. Minor

* Transfusion dependent; +20 (C>T); 2-745 [IVS-2-nt745 (C>G)]; CD8 (-AA); CD39 (C>T)

1804

CARDIAC MAGNETIC RESONANCE VERSUS ECHOCARDIOGRAPHY FOR THE ASSESSMENT OF CARDIAC VOLUMES AND FUNCTION IN THALASSEMIA INTERMEDIA PATIENTS

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Background. Cardiac Magnetic Resonance (CMR) and Echocardiography (US) are applied in parallel to thalassemia intermedia (TI) patients for cardiac evaluation and ongoing monitoring. It is important to know whether the results of each technique are interchangeable, and thereby how the results obtained utilizing one technique can be applied using another. These aspects are unexplored in TI. **Aims.** Tthe aim of this study was to evaluate the agreement of left ventricular volumes and ejection fraction (EF) by CMR and US. **Methods.** 74 TI patients (38 M and 36 F; mean age 36. 7±10. 9 yrs) patients were studied with both echo and CMR (1. 5T) within 3 months of each other. All TM patients were enrolled within the MIOT (Myocardial Iron Overload in Thalassemia) network where CMR exams were performed in 8 sites using homogeneous and validated procedures. Steady-state free procession cine images were acquired during 8-second breath-holds in sequential 8-mm short-axis slices from the atrio-ventricular ring to the apex to assess biventricular function parameters quantitatively in a standard way, using MASS® software. Previously demonstrated inter-center variability for the quantification of cardiac function was 6. 3%. Echocardiographic studies were carried out in 9 echo labs linked to the thalassemia centers. LV volumes and LVEF were measured by two-dimensional echocardiography using the biplane Simpson's formula. Paired-samples t-test or Wilcoxon test, correlation coefficient, intraclass correlation (ICC), and Bland & Altman plot were used to compare CMR and US parameters. **Results.** Table 1 shows the CMR and US parameters, quoted as mean ± standard deviation (SD), and the results of the comparison between the two techniques. All US volumes were significantly underestimated, especially the end-diastolic volume index, while the US EF was significantly higher than the CMR EF. The correlation between US and CMR end-diastolic and stroke volume indexes was significant, but with a very low coefficient; while the correlation for the ejection fraction was acceptable. The ICC was unsatisfactory for all volumes and good for the ejection fraction. The widest Bland Altman plot range was found for the end-diastolic volume index. **Conclusions.** In a moderately large CMR versus echocardiographic comparative study in TI patients, metrics of LV volume index and function showed significant systematic inter modality differences. In particular, the US volumes were systematically underestimated. This suggests that serial measurements of volumes and function in TI should be performed using the same method and if it is available the more reproducible CMR technique.

Table 1.

	LV EDVI (ml/m²)	LV ESVI (ml/m²)	LV SVI (ml/m²)	LV EF (%)
Values				
CMR	96.3 ± 17.9	36.6 ± 11.2	59.9 ± 11.8	62.0 ± 7.4
US	69.9 ± 18.1	26.2 ± 12.2	43.7 ± 12.9	63.7 ± 6.6
CMR-US				
Difference, mean ± SD	26.4 ± 22.3	10.4 ± 13.5	16.3 ± 15.8	-1.65 ± 7.7
P	<0.0001	<0.0001	<0.0001	0.051
Correlation, r (P-value)	0.229 (P=0.050)	0.182 (P=0.182)	0.394 (P=0.001)	0.473 (P<0.0001)
ICC	0.200	0.392	0.177	0.631
BA limits	-17.4 to 70.2	-16.0 to 36.8	-14.6 to 47.2	-15.7 to 12.4
BA range	87.6	52.8	61.8	28.1

EDVI= end diastolic volume index; ESVI= end systolic volume index; SVI= stroke volume index; EF= ejection fraction

1805

LEVELS OF ENDOGENOUS ERYTHROPOIETIN AND ERYTHROPOIETIN RECEPTOR EXPRESSION IN PATIENTS WITH LYMPHOMAS AND ANAEMIA

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Background. Erythropoietin (EPO) plays a key role in the adaptation of erythropoiesis to the metabolic needs for oxygen. Inadequate EPO production is one of the causes of anemia in patients with hematological malignancies and

solid tumors. **Aims.** We investigated the levels of serum erythropoietin (s-EPO), EPO receptors on the surface of erythrokariocytes bone marrow, we evaluated the efficacy of recombinant erythropoietin (rh-EPO) in patients with lymphoproliferative disease and anemias. **Methods.** We examined 109 patients with lymphoproliferative disorders and anemia (indolent and aggressive lymphoma, Hodgkin's disease, multiple myeloma). Serum erythropoietin (s-EPO) were studied in all patients. The level of expression of the erythropoietin receptor was investigated on the surface of erythropoietin - sensory cells of the bone marrow in 23 people. **Results.** The average level of EPO was 46,8 ± 8,16 mIU / ml in patients with indolent lymphomas. He was reduced (16,8 ± 2,55 mIU / ml) in 24 persons (60%). The average level of EPO was 36,9 ± 7,97 mIU / ml in patients with aggressive lymphomas. He was reduced (16,4 ± 2,35 mIU / ml) in 31 patients (79. 5%). The average level of EPO was 30,6 ± 8,07 mIU / ml in patients with Hodgkin's disease, decreased (20,9 ± 2,44 mIU / ml) in 14 (82. 3%). The average level of EPO was 38,8 ± 12,42 mIU / ml in patients with multiple myeloma, decreased (17,6 ± 3,91 mIU / mL) in 8 patients (61. 5%). Thus, the EPO has been inadequate in 70. 7% of surveyed patients in all groups of patients. The average number of EPO-R-positive (EPO-R +) cells 22,2 ± 4,23% in the bone marrow. The average number of erythrokariocytes was 24,1 ± 4,06% in the bone marrow. The number of EPO-R-positive cells (26,4 ± 4,81%) corresponds to the number erythrokariocytes in the bone marrow (26,4 ± 4,75%) only 73. 9% of cases. The number of EPO-R-positive cells (10,2 ± 3,93%) was significantly lower than the number of erythrokariocytes in the bone marrow (17,7 ± 4,26%) with 26. 1% of the patients (ρ <0,0001). **Conclusions.** Thus, these data indicate a significant role in the inadequate production of erythropoietin in the pathogenesis of anemia in patients with lymphomas. Reduced expression of erythropoietin receptors on the surface of erythroid cells in the bone marrow is one of the causes of anemia in some patients with lymphomas and anemia. This can cause resistance erythrokariocytes bone marrow to endogenous erythropoietin and the ineffectiveness of therapy with recombinant human erythropoietin. 6. Key words: erythropoietin, anemia, lymphoproliferative disease.

1806

NEONATAL SCREENING FOR HEMOGLOBINOPATHIES IN PUBLIC HOSPITALS ACROSS LEBANON

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Background. Hemoglobinopathies are among the commonest inherited blood disorders. The most prevalent hemoglobinopathies are thalassemias and sickle cell disease (SCD). SCD, the most frequent inherited monogenic disorder in the world, is characterised by hemolytic anemia, vasoocclusion and end organ dysfunction. The true prevalence of sickle cell disease and other abnormal hemoglobin variants in Lebanon is not known and is best determined through a neonatal screening program. Objectives:The primary objective of this neonatal screening was to determine the prevalence of SCD and other inherited hemoglobin disorders in Lebanon by examining 10,000 neonates from all public hospitals. Secondary study objectives included characterizing the demographic features of affected newborns and carriers and deciphering the exact genetic variants of abnormal hemoglobins detected. **Methods.** Neonates born in all public hospitals throughout Lebanon had each 3 drops of blood drawn on a filter paper in the first 3 days of their life. High pressure liquid chromatography (HPLC) was performed on these samples in a single central lab. HPLC results were forwarded to the principal investigator. The families of all neonates with abnormal HPLC results were contacted for rereconfirmation. Pertinent demographic information was collected. Parental consent was obtained prior to phlebotomy. **Results.** Among the 7248 neonates who underwent HPLC testing, 152 (2. 09%) had abnormal hemoglobins detected: 84. 21% had HbS, 8. 55% HbD; 3. 95% HbC and 3. 29% HbO Arab. Geographical clustering of these 152 neonates was noted in 2 regions: North Lebanon (50. 66%) and South Lebanon (30. 26%). Notably, the difference in the rate of consanguinity between parents of neonates with abnormal hemoglobins and those of neonates with normal hemoglobins was not statistically significant. **Summary and Conclusions.** This is the first nationwide neonatal screening campaign for inherited hemoglobin disorders aimed at targeting 10,000 neonates from all Lebanese territories. The prevalence rate of abnormal hemoglobins is comparable to that of beta thalassemia which has recently received significant national recognition in Lebanon and where affected patients receive optimal treatment in a single comprehensive center. This situation contrasts greatly with sickle cell disease where some patients don't have access to modern treatment due to lack of national awareness about this disease and to suboptimal and delayed diagnosis at times. The geographical clustering of abnormal hemoglobins in the Northern and Southern regions requires further investigation and tracing of the ethnic origin of affected neonates residing in these 2 regions. Additionally, awareness campaigns about hemoglobinopathies particularly sickle cell disease emphasizing the benefits of premarital carrier detection, neonatal screening and early implementation of modern treatment, need to be initiated in these areas of high prevalence. Moreover, the lack of significance of consanguineous marriage to the carrier status of the abnormal gene was an unexpected finding and may indicate a high gene frequency of the sickle gene and genes for the other abnormal hemoglobins . This study still aims at screening an additional 2752 neonates to reach its 10. 000 target. Moreover, DNA has been collected for mutational analysis of afflicted individuals and detailed genetic studies are currently being established.

1807

CLINICAL APPLICATION OF RECOMBINANT ERYTHROPOIETIN IN β-THALASSAEMIA INTERMEDIA

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Background. The basic method of anemia correction at β-thalassemia is blood transfusion. However, together with high therapeutic effect this method has serious complications: transfusion transmitted infections, alloimminization, iron overload. Augmentation of fetal hemoglobin represents a direct approach to reduce the globin chain imbalance and ineffective erythropoiesis characteristic of β-thalassemia without blood transfusions. 5-Azacytidine, cytarabine, vinblastine, hydroxyurea, and arginine butyrate have been demonstrated to augment HbF synthesis in animals and in patients with β-hemoglobinopathies. **Aims.** The studies objective is to research the efficacy of recombinant erythropoietin (epoetin alfa) as alternative method of treatment β-thalassemia intermedia. **Methods.** Study involved 57 patients with β-thalassemia intermedia (22 women and 35 men). An informed consent form was signed by the patients or their parents. Control group of 20 patients with β-thalassemia intermedia didn't receive recombinant erythropoietin (rEPO). In all observed patients was defined levels of hemoglobin (Hb), red blood cells (RBC), hematocrite (Ht), erythrocyte indexes (MCV, MCH, MCHC), hemoglobin fractions (HbA, HbA2, HbF), serum iron, serum ferritin, serum erythropoietin before and after administrated rEPO. **Results.** In all patients the levels of erythropoietin was elevated. All patients received rEPO (during 16 weeks) at the dose - 10000 IU subcutaneously 3 times a week. Each 8 weeks treatment's efficacy was estimated. The study showed that, in 44 patients receiving rEPO, Hb level increased more than 2 g/dl, in 11 patients this rate was between 1 g/dl - 2 g/dl. But in two patients increasing of Hb level wasn't observed. The majority of patients reported increse of fetal hemoglobin (HbF) levels. Iindicators of serum iron and serum ferritin are reliably reduced. Blood transfusion wasn't requaired during treatment with rEPO and consequently it avoided iron overload. At the same time, regardless of treatment's effectiveness, even in cases, where Hb level wasn't increased, improvement of patients' cheerfulness and good mood were revealed. **Conclusions.** Thereby, we can come to the conclusion that rEPO prescriptions had high effectiveness and diminished the requirement of patients with β-thalassemia intermedia to hemotransfusion and iron-chelating drugs, and improving the quality of life of patients.

1808

GENDER BUT NOT IRON OVERLOAD INCREASES THE RISK OF BONE DISEASE IN PATIENTS WITH THALASSEMIA MAJOR

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Introduction. Bone disease is a recognized complication in patients with thalassemia major. The disease is a risk factor. We hypothesized that patients with increasing iron overload have higher risk of osteoporosis. **Aims.** We planned to estimate the prevalence of osteopenia and osteoporosis in patients with thalassemia major receiving regular blood transfusions and iron chelation therapy. We also planned to estimate the impact of iron overload on the prevalence of osteopenia and osteoporosis in these patients. **Methods.** Bone density measurements are done after the age of 20 years as a routine part of thalassemia management in our institution. We retrospectively analyzed 76 patients with thalassemia major who were at least 20 years old. We classified them into the following categories: osteoporosis (lumbar and/or femoral Z scores of -2. 5 or less) and osteopenia (lumbar and/or femoral Z scores of -1. 0 or less and >-2. 5). We collected data on age, gender, height, weight, ferritin level when scan was done, diabetes status and hypoparathyrodism. We used multivariable ordinal logistic regression to estimate the impact and adjust for gender, age, body mass index (BMI) and ferritin. **Results.** We evaluated 76 consecutive patients with thalassemia major treated at our tertiary care center.

There were 37 females and 39 males with a mean age of 22. 8 years (standard deviation [SD] 3. 5, range 20-33). The mean weight and height were 55. 5 kg (SD 12. 1, range 36. 4-91. 5) and 1. 6 meter (SD 0. 1, range 1. 4-1. 83) respectively. The mean BMI was 22 (SD 4, range 15-35). The median ferritin level was 1550 ng/ml (not normally distributed, range 50-14538). Fourteen and 10 patients had non-insulin and insulin dependent diabetes mellitus respectively. Nine patients had hypoparathyrodism. Osteopenia and osteoporosis was seen in 30 (39%, 95% confidence interval [CI] 28-51) and 39 patients (51%, 95% CI 40-63) respectively. In the multivariable ordinal logistic regression, only gender (male vs. female) was statistically significant for predicting bone disease (odds ratio [OR] 3. 4, 95% CI 1. 3-8. 7, p=0. 012). Ferritin level did not impact on the development of bone disease (OR 1. 0, p=0. 193). **Conclusions.** Bone disease (osteopenia and osteoporosis) is very common in patients with thalassemia major on regular blood transfusion despite being on iron chelation therapy. This may be due to the fact that these patients are living longer. Gender but not iron overload increases the risk of bone disease. A larger confirmatory study is needed.

1809

HEMOGLOBIN LEVEL AS A PREDICTOR OF HEMOLYTIC CRISES IN PATIENTS WITH SICKLE CELL TRAIT

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Background. homozygous hemoglobinopathy S is a serious disease affecting all organs, with high mortality rates. To be a carrier of sickle cell trait is not a disease, although it has been reported that some of them may develop clinical feature of sickle cell disease such as vaso-occlusive crises and acute chest syndrome. **Aims.** to assess whether sickle cell trait carriers develop clinical manifestations of sickle cell disease. To determine whether there is any correlation between biological parameters and the typical complications of sickle cell disease in carriers. **Methods.** this retrospective study recruited patients with structural hemoglobinopathy S from the spanish health district of Zaragoza (367,110 inhabitants), between june 2006 and december 2011. Patients variables assessed at diagnosis: total leukocyte count, hemoglobin level, hematocrit, mean corpuscular volume, percentage of HbS and HbF. Epidemiological and clinical parameters, such as presence or absence of vaso-occlusive crisis, hemolytic crisis, acute chest syndrome, infections, hospital admissions related to clinical manifestations of sickle cell disease and thrombotic events were also assessed. **Results.** in 128 patients hemoglobinopathy S were detected. 105 patients were HbA/HbS (sickle cell trait). 52 men (49. 5%) and 53 women (50. 5%). 5. 7% were of European origin, 37. 1% American, 54. 3% African and 2. 9% Hindu. The median age at diagnosis was 31 years (1-79). 11 patients (10. 5%) had at least one vaso-oclussive crisis, 21 patients (20%) had an infectious process, requiring admission to hospital, 3 patients (2. 9%) a thrombotic event, 3 patients (2. 9%) at least one hemolytic crisis. No acute chest syndrome was found. Mean number of hospitalization days related to complications of sickle cell anemia was 3. 14 days (1. 36 to 4. 92), without related mortality. HbA/HbS patients with a leukocyte count over $10x10^9$/L had a 4 times more risk of developing vaso-occlusive crises than those with lower count, OR 4. 03 (0. 88 to 18. 28, p = 0. 055). On the other hand, the sickle cell trait carriers who had a hemoglobin (Hb) less than or equal to 12 g/dL at diagnosis had more hemolytic crisis (3 events vs 0, P = 0. 039). No statistically significant correlation with other laboratory parameters and clinical manifestations was found. **Summary and Conclusions.** an Hb level of less than or equal to 12 is significantly correlated with a higher risk of hemolytic crisis in patients with sickle cell trait. In our experience this parameter as well other laboratory markers could be useful to predict complications in this setting.

1810

DEFERASIROX FOR THE TREATMENT OF CHRONIC IRON OVERLOAD IN PEDIATRIC ONCOLOGY PATIENTS - A SINGLE CENTER EXPERIENCE

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Background. Iron chelation has not been studied adequately in the pediatric oncology population, despite the fact that many patients are heavily iron overloaded secondary to blood transfusions administered due to the bone marrow suppressing effects of chemotherapy. Hemosiderosis can add to the oxidative stress and multiple endocrine abnormalities that these patients are already experiencing. Deferasirox is an oral iron chelator that can potentially be used to improve the morbidity and mortality in this patient group. **Aims.** To report the efficacy and safety of deferasirox in the clinical setting of an academic pediatric oncology center. **Methods.** Thirteen patients (7 male, 6 female, mean age 11. 04 years, SD=3. 99, range=2. 37-16. 31) with pediatric malignancies in remission and iron overload secondary to multiple blood transfusions during chemotherapy were monitored prior to deferasirox administration for an average of 26 months (SD=17, range 2-45). MRI iron studies were performed prior to initiation of chelation therapy. Deferasirox was administered for an average of 6 months (SD=4. 5, range=0. 3-18. 2) to children that were either severely iron overloaded as assessed by MRI, or had ferritin levels persistently >2500 µg/dl. Dosage was 20 mg/kg/day for 10 patients and 30 mg/kg/day for 3 patients. All patients had completed chemotherapy, were chelation-naïve, and did not receive blood transfusions during the entire follow-up period. Ferritin, urea, creatinine, transaminases, and bilirubin were recorded at 4-8 week intervals. The rate of change in ferritin before and during deferasirox treatment was assessed using piecewise linear mixed effects models. **Results.** Patient characteristics and MRI findings are presented in Table 1. Two patients discontinued deferasirox shortly after initiation of treatment due to skin rash. One patient reported mild gastrointestinal disturbances that subsided after the first week of treatment. One patient presented with acute renal failure 4. 8 months after treatment initiation and fully recovered with supportive measures within one week after cessation of deferasirox. Other factors may also have contributed to renal impairment, including concomitant medications and poor fluid intake. The mean monthly rate of change in ferritin levels was -10. 8 µg/l before initiation of treatment (95% CI: -19. 8 to -1. 8, p=0. 02) and -93. 6 µg/l during deferasirox treatment (95% CI: -118. 1 to -69. 1, p<0. 001). The difference in the monthly rate of change in ferritin levels before and after treatment initiation was -82. 8 µg/l (95% CI: -111. 6 to -53. 9, p<0. 001). **Summary and Conclusions.** Deferasirox was effective in reducing the iron burden in pediatric oncology patients. The adverse effects were easily monitored and managed in the clinical setting. This oral iron chelator could prove to be a useful tool in the field of pediatric oncology. Further research is needed to accurately define its use.

Table 1. Patient characteristics and MRI findings.

Diagnosis, n	medulloblastoma 6, glioblastoma multiforme 1, anaplastic astrocytoma 1, anaplastic ependymoma 1, PNET 1, ALL 2, hepatoblastoma 1
Baseline Ferritin, µg/L (SD)	1541 (858)
Range	687-3500
Liver Iron Overload, n (%)	
Mild	2/13 (15%)
Moderate	3/13 (23%)
Severe	8/13 (62%)
Liver T2*, msec (SD), Normal>19.1	5.8 (2.0)
Range	3.8-9.4
Liver Iron Concentration +/-50 µmol/g (SD), Normal<36	205 (81)
Range	90-350
Cardiac Iron Overload	0/13
Cardiac T2*, msec (SD), Normal>22	34.1 (5.8)
Range	25.6-43

1811

FRUCTOSAMINE AS GLUCEMIC MARKER IN PATIENTS WITH THALASSEMIA

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Purpose. The measurement of fructosamine in patients with thalassemia and diabetes mellitus type I and II and its credibility as a glucemic marker in comparison with the measured HbA_1c in these patients. **Methods.** A prospective case control study was conducted in the Laboratory of Haematology in St. Andreas Hospital of Patras, Greece, during the year 2010 including patients with thalassemia and diabetes mellitus type I and II undergoing treatment. None of the patient suffered from hyperthyroidism, hypercholesteremia or liver disease and all patients had normal values of serum total protein and albumin. Both HbA_1c and fructosamine as glucemic markers were measured. Measurements of HbA_1c were made using the Menarini HPLC method(High perform-

ance liquid chromatography-HA 8160) and those of fructosamine using the ROCHE liquid chromatography method (Integra 400). **Results.** The study included 128 type I and II diabetes mellitus patients (mean age 33. 4 +/-8. 9 years) and 41 controls. Among the diabetic patients, 32% were patients with thalassemia major and 33% were patients with thalassemia minor. We observed significantly higher mean levels HbA1c and fructosamine in patients with thalassemia and diabetes mellitus type I and type II. Was calculated mean level HbA1c 7. 1% (SD 1. 51,) and mean level fructosamine 292. 43mmol/l (SD 64. 70,) p< 0. 0001. **Conclusions.** In patients with thalassemia and diabetes mellitus a routine measurement of fructosamine every 45 days is required, in order to evaluate the adequacy of the diabetes treatment. This specific test is preferable and corresponds to the HbA_1c measurement, especially in patients with thalassemia major, as HbA_1c (due to the pathophysiology of this hemoglobinopathy and because of the frequent blood transfusions) underestimates the real mean level of glucemia.

1812

IRON DEFICIENCY ANEMIA AS THE INITIAL MANIFESTATION OF UNDERLYING MALIGNANCIES

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Background. Iron deficiency anemia (IDA) is a manifestation of many underlying diseases. **Aims.** We would like to investigate the frequency of, risk factors for, and prognosis of IDA as the initial manifestation of underlying malignancies over a 12-year period in Taiwan. **Methods.** We extracted 5,512 patients with iron deficiency anemia from a nationwide health registry in Taiwan during the period 1997-2008. The frequency for IDA as the initial manifestation of underlying malignancies was determined. Standardized incidence ratios (SIRs) of cancers were calculated to compare the cancer incidence of IDA patients to general population. The specific types of cancer were addressed. **Results.** A total of 319 (5. 79%) IDA patients developed cancer within the study period (SIR 2. 46; 95% confidence interval [CI] 2. 20-2. 75). One hundred and fifty-two (2. 76%) patients who developed cancer within one year presented with IDA as the initial manifestation of underlying malignancies (SIR 5. 88; 95% CI 4. 98-6. 89). The risks of head and neck (SIR 1. 66, 95% CI 0. 83-2. 96), stomach (SIR 4. 68, 95% CI 3. 20-6. 61), colon and rectum (SIR 5. 23, 95% CI 4. 23-6. 40), liver and biliary (SIR 2. 73, 95% CI 1. 94-3. 73), pancreas (SIR 2. 86, 95% CI 1. 05-6. 22), uterus (SIR 3. 52, 95% CI 1. 76-6. 29), bladder (SIR 4. 14, 95% CI 2. 37-6. 73), kidney (SIR 3. 70, 95% CI 1. 85-6. 62), and hematologic malignancies (SIR 3. 46, 95% CI 2. 05-5. 47) increased. Among all age and sex groups, males aged 18-39 had highest SIR. **Conclusions.** Patients who presented with IDA had six-fold higher risk of cancer development within the first year. IDA might be the initial manifestation of underlying malignancies, especially in young males.

1813

ARE CARDIAC R2* VALUES DEPENDENT ON THE USED IMAGE ANALYSIS APPROACH?

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Background. Cardiovascular magnetic resonance (CMR) R2* has become a widely used technique for monitoring cardiac iron overload in patients with different types of hemoglobinopathies. The R2* value is obtained by fitting the CMR signal at different echo times (TEs) to an appropriate decay model. Patients with heavy cardiac iron burden (T2* < 10 ms) exhibit rapid signal, leading to a plateau in the later echo images. Two approaches have been used to address this. The first one (truncation model) consists in discarding the late 'plateau' points and fitting the remaining ones with a single exponential model. The second approach is to fit the signal to an exponential decay plus a constant offset. **Aims.** Our aim was to determine whether systematic differences were present between R2* values obtained with these two approaches. **Methods.** Single-center cohorts were used to compare black blood and bright sequences separately and a multi-center cohort of mixed bright and black blood studies was used to compare robustness and generalizability of the comparison. The R2* value within a region of interest (ROI) drawn in mid-ventricular septum was assessed by the same operator using each of the two methods in turn. Truncated exponential estimates were calculated with CMRTools that uses a truncated, single ROI method ($R2^*_{CMRTools}$). Exponential plus constant (Exp-C) estimates were calculated using a rapid pseudo-pixelwise (PPW) implementation written in MATLAB. A distribution of R2* values within the septal ROI was produced and the mean and median from this distribution were obtained ($R2^*_{PPW\text{-}mean}$ and $R2^*_{PPW\text{-}median}$, respectively). To distinguish whether differences in measured R2* values resulted from the underlying fitting model or from the use of a PPW rather than a region-based approach, we performed Exp-C fits to a single ROI ($R2^*_{PPW\text{-}ROI\_based}$). **Results.** Table 1 shows the results for the two methods. No differences could be distinguished based upon whether a white or black blood sequence was examined. The two fitting algorithms gave similar R2* values, with R-squared values exceeding 0. 997 and CoV of 3-4%, indicating excellent stability of both techniques. *Results using the PPW method yielded a small systematic bias (~3%) that became apparent in patients with severe iron deposition (R2* >100 Hz). This disparity, however, disappeared when Exp+C fitting was used on a single ROI suggesting that the use of pixel-wise mapping was responsible for 3% bias, rather than the fitting model.* In the multicenter cohort the strong agreement between R2* values obtained with the two fitting approaches was reconfirmed, proving high generalizability of our results. **Conclusions.** Cardiac R2* values are independent of the signal model used for its calculation over clinically relevant ranges; pixelwise fitting generate insignificantly greater R2* estimates at high iron concentrations. The overall variability among the considered techniques is exceeding small allowing clinicians to compare results among centers using these disparate approaches with confidence.

Table 1.

	Paired t-test		Regression Analysis				Bland Altman		CoV (%)
	Mean Values (Hz)	P	Slope	P for Slope ≠1	Intercept (Hz)	R-squared	Mean diff (Hz)	Limits (Hz)	
a) First single-center cohort: black blood images (N=42)									
$R2^*_{Iron\text{-}mean}$ vs $R2^*_{CMRTools}$	48.5±54.7 vs 48.2±53.1	0.945	1.031±0.005	<0.0001	1.176±0.379	0.999	0.3	4.4 to 5.1	3.53
$R2^*_{Iron\text{-}median}$ vs $R2^*_{CMRTools}$	48.9±55.0 vs 48.2±53.1	0.258	1.036±0.007	<0.0001	-1.007±0.473	0.998	0.7	- 5.0 to 6.5	4.37
$R2^*_{Iron\text{-}ROI\_based}$ vs $R2^*_{CMRTools}$	48.9±52.7 vs 48.2±53.1	0.063	0.993±0.007	0.336	1.058± 0.512	0.998	0.7	-4.1 to 5.5	3.66
b) Second single-center cohort: bright blood images (N=70)									
$R2^*_{Iron\text{-}mean}$ vs $R2^*_{CMRTools}$	47.6±37.9 vs 47.2±36.9	0.088	1.025±0.005	<0.0001	-0.741±0.300	0.998	0.4	-3.1 to 3.9	2.70
$R2^*_{Iron\text{-}median}$ vs $R2^*_{CMRTools}$	47.7±38.2 vs 47.2±36.9	0.085	1.035± 006	<0.0001	-1.190±0.373	0.998	0.4	-4.0 to 4.9	3.45
$R2^*_{Iron\text{-}ROI\_based}$ vs $R2^*_{CMRTools}$	47.5±37.4 vs 47.2±36.9	0.050	1.013±0.004	0.002	-0.245±0.247	0.999	0.4	-2.3 to 3.0	2.06
c) Multi-center cohort (N=62)									
$R2^*_{Iron\text{-}mean}$ vs $R2^*_{CMRTools}$	43.5±22.6 vs 43.8±22.7	0.250	0.989±0.008	0.148	0.108±0.372	0.997	-0.4	-3.0 to 2.3	2.25
$R2^*_{Iron\text{-}median}$ vs $R2^*_{CMRTools}$	43.7±22.4 vs 43.8±22.7	0.989	0.982±0.007	0.015	0.705±0.359	0.997	-0.1	-2.7 to 2.6	2.25
$R2^*_{Iron\text{-}ROI\_based}$ vs $R2^*_{CMRTools}$	44.0±22.5 vs 43.8±22.7	0.207	0.989±0.007	0.131	0.681±0.352	0.997	0.2	-2.3 to 2.7	2.07

1814

ASSESSMENT OF SERUM ERYTHROPOIETIN AND ANTI-ERYTHROPOIETIN ANTIBODIES LEVELS IN ANEMIC CHRONIC HCV INFECTED PATIENTS

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Background. Chronic anaemia frequently associate with chronic HCV infection is one of the potential factors predicting the risk of morbidity and mortality; and may have a negative impact on the current standard treatment for HCV infection, the etiologic role of endogenous erythropoietin and anti-erythropoietin is controversial. **Design of the Study.** A descriptive cross sectional study **Objectives of the Study.** To assess levels of the circulating erythropoietin and anti-erythropoietin antibodies in HCV positive chronic liver disease patients with chronic anemia. **The study Population.** A total of eighty chronic HCV infected patients were included. *The study group:* Sixty Anti-HCV positive patients with chronic anemia (54 (67. 5%) males and 26 (32. 5%) females), they were subdivided into three subgroups according to Child-Pugh classification. *the Control Group*: twenty Anti-HCV positive patients with normal peripheral hemogram. **Methods.** Medical histories, clinical examination, complete liver function tests, test for HCV antibodies and detection of HCV RNA by RT-PCR were carried out. Complete blood picture & reticulocytic count, serum ferritin, assessment and quantification of serum Erythropoietin and Anti-Erythropoietin antibodies using ELISA technique. **Results.** Statistical analysis of the obtained data showed a statistically significant decrease in the mean values of Hb concentration, hematocrit values, and reticulocytic count in child B & child C subgroups compared to that of child A subgroup. There is statistically significant increase among the mean values of Serum ferritin among different child classes of anemic group child A (144 ± 63), child B (380 ± 76) and child C (611 ± 70)

(p < 0. 001). The mean values of serum erythropoietin in the anemic group (50. 48 ± 44. 7) were significantly higher than that of non anemic group (9. 3 ± 6. 8) (p0<001). There is statistically significant difference among the mean values of Serum erythropoietin among different child classes of anemic group child A (27. 25 ± 10. 7), child B (42. 65 ± 25. 9) and child C (66. 82 ± 63. 8). Anti-erythropoietin antibodies were positive only in 7 patients (11. 7%) of anemic group child C class. There is statistically significant negative correlation between erythropoietin and HB (r = -0. 5, p < 0. 001), negative correlation between erythropoietin and hematocrit (r = -0. 3, p < 0. 01), a positive correlation between erythropoietin and serum ferritin (r = -0. 6, p < 0. 001), **Conclusions and Recommendations.** data obtained in the present study including high ferritin, increased endogenous erythropoietin and low frequency of anti-erythropoietin in the anemic HCV infected patients could indicate failure of iron utilization during erythropoiesis, defective appropriate response of the hematopoietic stem cells (target tissue irresponsiveness). The role of exogenous recombinant erythropoietin in circumventing this irresponsiveness.

1815

SICKLE CELL ANAEMIA IN NIGERIAN CHILDREN: PARENTAL KNOWLEDGE AND LABORATORY Results

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Background. Sickle cell disease (SCD) is the most common inherited genetic disorder in sub Saharan Africa and it is associated with early mortality and lifelong morbidity. Early diagnosis is essential in enabling parents / carers to institute appropriate care and preventive therapy. Alkaline gel electrophoresis (paper chromatography) is widely used in SCD screening in developing countries however this method is insensitive in the first six months of life and has been associated with inadvertent errors. High Performance Liquid Chromatography (HPLC) is a reliable tool for newborn SCD screening. **Aims.** The aim of study was to ascertain parental knowledge / perception of their offspring's haemoglobin phenotype prior to testing and actual validated laboratory test results. **Methods.** Children aged less than 5 years of age across several communities inKaduna and Katsina states (North West zone) and the Federal Capital Territory of Nigeria were offered free screening for sickle cell anaemia during June 2010- March 2011. The programme was part of a research project on establishing a sustainable cohort of children with sickle cell haemoglobinopathy for the purpose of providing them with comprehensive health care. Following pre-test counselling, informed consent for testing was obtained from the parent or guardian of child before testing. A short questionnaire was then administered to obtain basic demographic information and parental knowledge of their child's previous haemoglobinopathy screening result, if applicable. The information given by the parent or guardian was corroborated with the results obtained from HPLC testing performed as part of the screening programme. This study was approved by the Institutional Review Boards of the Federal Capital Territory (FCT),KadunaState andKatsina State,Nigeria and Institutional Review Board of Michigan State University, United States of America. **Results.** A total of 11,684 children aged less than 5 years of age were enrolled on to the screening programme. The parents/guardians of 199 (2%) of these children indicated on questioning that their child had previously been tested for SCD. However the parents were not aware of the test results in 57 (28%) children; 13 of these children were found to have SCD. Of the 142 (71%) children, whose parents claimed prior knowledge of their haemoglobin phenotype, the results given by the parents were incorrect in 28 (20%) children. We identified 283 new cases of SCD in 11, 485 children who had not been previously tested. **Conclusions.** There is the need to promote public awareness and provide education on SCD including the benefit of early diagnosis. Improvement and monitoring of laboratory diagnostic services are urgently required in order to provide accurate diagnostic test results to patients. In addition this must be supported by appropriate genetic counselling and referral for care. Availability of written results following testing is strongly advocated as part of the primary health care in this setting, given the prevalence of SCD and the potential impact of this knowledge on patient care.

			Lab results: genotype				Total
			AA	AS	SS	Other	%column
Self-Report: test results	AA		4 44.40%	3 33.30%	2 22.20%	0 0.00%	**9** **4.52%**
	AS		4 26.70%	5 33.30%	6 40.00%	0 0.00%	**15** **7.54%**
	SS		8 6.80%	4 3.40%	105 89.70%	0 0.00%	**117** **58.79%**
	SC		0 0.00%	0 0.00%	1 100.00%	0 0.00%	**1** **0.50%**
	Don't Know		34 59.60%	9 15.80%	13 22.80%	1 1.80%	**57** **28.60%**
	Total		**50** **25.10%**	**21** **10.60%**	**127** **63.80%**	**1** **0.50%**	**199** **100.00%**

1816

IS IT NECESSARY TO MONITOR PNEUMOCOCCAL VACCINE TITRES IN SICKLE CELL ANAEMIA PATIENTS EVERY YEAR?

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Background. Patients with sickle cell anaemia are hyposplenic and hence more prone for pneumococcal infection. The risk of this can be reduced by the administration of specific pneumococcal vaccines. The current Irish immunisation schedule recommends 3 doses of Prevnar® in children less than 1 year. Children with Sickle cell disease above 2 years are given 1 dose of pneumovax® and repeated in 3-5 years. Pneumococcal IgG titres more than 20u/ml is considered protective against Pneumococcal infection. Booster doses are recommended if the titres fall below this level. **Aims.** To determine the rate and efficacy of pneumococcal vaccine in children with Sickle cell disease and to assess the need for revaccination. **Methods.** All patients with Sickle cell disease received Pneumococcal vaccination as per Immunisation guidelines following diagnosis. Titres were measured on an yearly basis. An audit of sickle cell patients who received Pneumococcal vaccination over 5 years (2003 - 2008) was undertaken. Pneumococcal vaccine serology was obtained from the hospital laboratory database and vaccination details from patient records. **Results.** 232 patients were included in the study of which 120 were males and 112 females. Age varied from 2to 21 years. In 198 (85%) patients Pneumococcal titres remained above 20 u/ml at the end of the study period although the titres showed a gradual fall over the years. Pneumococcal titres fell below the protective levels in 34 patients (15%) on follow up. In 16 patients rate of fall occurred in the 2nd year,10 in 3rd year, 1 in the 4 th year and 2 in the 5 th year . The lowest trough levels was 10 u/ml. 4 patients failed to mount an adequate immune response. **Conclusions and Summary.** Pneumococcal vaccine titres were found to fall each year in the cohort of sickle cell patients studied . In 15% of patients the levels fell below the protective levels and needed booster doses. Hence the titres should monitored on an yearly basis.

1817

DIAGNOSTIC PERFORMANCE OF THE RETICULOCYTE HAEMOGLOBIN EQUIVALENT FOR FUNCTIONAL IRON-DEFICIENCY ANEMIA IN HAEMODIALYSIS PATIENTS

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Introduction. The administration of recombinant human erythropoietin (rhEPO) in patients undergoing chronic haemodialysis increases haematocrit levels but the increased demand of iron for erythropoiesis leads to functional iron deficiency anaemia. The usefulness of reticulocyte haemoglobin equivalent (RET-He) measurement in peripheral blood samples is studied for the diagnosis of iron deficiency anaemia in children and in elderly. **Aims.** The aim was to determine the RET-He values, that characterize functional iron deficiency anaemia in chronic haemodialysis patients, based on the classical method of transferrin saturation measurement. **Materials.** Seventy five patients, undergoing chronic haemodialysis treatment at Nephrology Department of AHEPA University Hospital, were considered for inclusion in this study. All patients received ery-

thropoietin, and iron was administered intravenously to maintain the haemoglobin level between 10 and 12 mg/dL. A Roche/Hitachi Modular System P800 was used to determine the concentrations of iron using spectrophotometric analysis, and transferrin using immunoturbidimetric assay. Transferrin saturation (TSAT) was calculated as follows: serum iron (µg/dl) x 100 divided by transferrin (mg/dl) x 1. 25. RET-He was measured with the automated hematology analyzer Sysmex XE-5000. Receiver operator characteristic curve (ROC) was constructed to evaluate the diagnostic performance of the Ret-He for iron-deficiency anemia. **Results.** Mean values ± standard deviation of TSAT and Ret-he were 35. 8±20. 5%, and 31. 4±3. 8pg, respectively. A statistically significant correlation was found between Ret-he and TSAT (r=0. 265, p=0. 031). Transferrin saturation below 20% constitutes criterion for iron-deficiency diagnosis in hemodialysis patients. ROC curve, after the maximal product of sensitivity x specificity, revealed that iron deficiency could be diagnosed by using a Ret-He cutoff level of 30. 7 pg, with a sensitivity of 76. 1%, and a specificity of 75%. The area under the curve was 0. 795 with 95% confidence interval, 0. 669-0. 921, with p<0. 0001. **Conclusions.** Our study showed a good diagnostic efficacy of RET-He for evaluation of patients requiring iron support. RET-He could be a useful marker to guide and monitor iron treatment in dialysis patients.

1818

PREOPERATIVE USE OF RECOMBINANT HUMAN ERYTHROPOIETIN AND IRON SUCROSE TO DECREASE BLOOD TRANSFUSIONS IN PULMONARY TUBERCULOSIS PATIENTS AFTER SURGERY (PILOT STUDY)

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Background. Tuberculosis is a chronic, infectious disease that primarily attacks the lungs. In cases of strong drug-resistant strains of *Mycobacteria tuberculosis* and some others, the patients may undergo operative intervention to remove the infected areas. In these cases postoperative anemia are usual. A retrospective review of 60 operated patients indicated that approximately 50% of them had anemia and 20% of anemic patients received blood transfusions after surgery immediately. Postoperative anemia is harmful for these patients as it impairs rehabilitation and hospitalization time them. **Aims.** To evaluate the ability of preoperative use of recombinant human erythropoietin (rHuEPO, epoetin alfa) and iron sucrose intravenously to decrease allogenic blood transfusions and the associated risks in patients undergoing surgical treatment of pulmonary tuberculosis. **Methods.** The trial protocol was approved by the Ethical Committee of Federal Research Center for Pediatric Hematology, Oncology and Immunology named D. Rogachova, Moscow, Russia. Nine pulmonary tuberculosis patients aged 24 to 53 years those who had high risk to receive blood transfusions after planned operative intervention were enrolled into the trial after providing informed consent. Mean Hb values before rHuEPO administration were 125. 3±10. 53 g/L, mean serum ferritin levels - 33. 2±21. 14 µg/L. The trial medication 600 IU epoetin-alfa/kg body weight per week was administered subcutaneously on preoperative days 14, 7, and day of operation. The exclusion criteria were diastolic blood pressure greater than 100 mm Hg, hematocrit level greater than 0. 45, Hb level over 140 g/l, platelet count greater than 500×10^9/L. All patients received 100 mg (5 ml) iron sucrose intravenously twice weekly during two weeks before operation and one more on operation's day. Blood loss was quantified by weighing sponges and measuring suction volume intraoperatively and subtracting the volume of the irrigation fluid. After operative intervention we evaluated Hb concentrations and serum ferritin levels during four weeks and blood transfusion's volume and quantity. **Results.** Perioperative blood loss were from 165 to 1350 mL (403. 1±249. 96 mL). None of all patients who received rHuEPO and iron sucrose has not required perioperative blood transfusions. The mean Hb concentrations in operated patients in 1 day after surgery were 122. 0±14,82 g/L. Mean serum ferritin levels in patients to four week of observation composed 87. 7±42. 98 µg/L. No influence of epoetin therapy on blood pressure, laboratory safety variables, or the frequency of specific adverse events was observed. **Conclusions.** This study suggests that in pulmonary tuberculosis patients, who has high risk to receive blood transfusions after planned operative intervention, preoperative use of epoetin alfa combined with iron sucrose treatment is an efficient method to decrease perioperative transfusion requirements and to increase perioperative Hb concentration. Moreover, we expect that preoperative epoetin alfa treatment might also improve rehabilitation of patients after surgery and enable reduction in hospitalization time.

1819

ERYTHROPOIETIN PROVIDES A USEFUL STRATEGY FOR TREATING PREOPERATIVE ANEMIA IN PLANNED ELECTIVE ORTHOPAEDIC SURGERY

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Background. Major orthopaedic surgery can require transfusion of significant quantities of allogeneic blood, which carries risks of infection, allergy and incompatibility. Furthermore, reduced supply of blood components and high costs of blood preservation impact on health economics. Recent progress in the development of a blood-based assay for prion infection by variant Creutzfeldt-Jakob Disease (vCJD) may reduce infection risk, but correspondingly may further reduce the availability of red cells for transfusion. **Aims.** To evaluate erythropoietin as a strategy for correcting preoperative anemia before major orthopaedic surgery. **Methods.** Erythropoietin therapy was considered for patients with moderate anemia scheduled for planned primary total hip replacement (THR) or revision THR or total knee replacement (TKR). Data was also collected from one patient who received erythropoietin regimens on two occasions prior to right acetabular revision procedure, because of religious objections to a blood transfusion. Patients were assessed for haemoglobin (Hb) and haematinics 21 days before surgery, and weekly thereafter. Patients with Hb 10-13 g/dL were administered weekly erythropoietin subcutaneously. Two patients were included at the clinical discretion of the investigator who had initial Hb 9. 8 g/dL and 9. 9 g/dL respectively. The protocol encouraged weekly erythropoietin from 21 days before surgery unless Hb >14 g/dL. Erythropoietin was not administered on the day of surgery except in two patients who joined the study 14 days before surgery. All patients received postoperative thromboprophylaxis. **Results.** We identified 30 episodes of care for 29 patients with a mean age of 73. 4 years (48 to 88 years) and pre-erythropoietin mean Hb of 11. 3 g/dL. 22 episodes of care achieved three erythropoietin injections at weekly intervals, and 8 episodes of care achieved only two erythropoietin injections. The mean Hb of all patient episodes on the day of surgery was 12. 9 g/dL. For male patients, the post-erythropoietin mean Hb was 12. 7 g/dL and 60% (n=5) had Hb within normal range according to the WHO criteria. For female patients, the post-erythropoietin mean Hb was 12. 9 g/dL and 88% (n=25) had Hb within normal range. Surgery without blood transfusion was performed in 90% (27 of 30) of episodes of care. Of the remaining three episodes, blood transfusion was required in only one, due to severe perioperative haemorrhage unrelated to treatment. **Conclusions.** Transfusion risk and paucity of blood components require imaginative solutions, particularly in the UK where vCJD risk is germane. Our results from a small cohort of patients confirms recent findings from the European Erythropoietineitin Alfa Surgery Trial (EEST) that administration of preoperative erythropoietin is associated with a low transfusion rate and few complications amongst a selected population group. Interestingly, in contrast with the EEST, our study did not administer erythropoietin on the day of surgery for most episodes of care. Further studies should be undertaken to establish an optimal erythropoietin regimen, including evaluation of the longer acting Erythropoiesis Stimulating Agents.

1820

WHEN WATER DOESN'T CLEAR THE SMUT FROM THE SMOKE: SECONDARY POLYGLOBULIA CAUSED BY ACCIDENTAL CO-INTOXICATION RELATED TO EXCESSIVE WATER-PIPE SMOKING

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Background. Tobacco smoking with a water-pipe (Shisha) is increasingly popular among young people and adolescents. Burning charcoal is used to heat flavoured tobacco, which is inhaled with a hose by drawing the smoke through a water-bowl. The proponents of water-pipe smoking claim that tobacco inhalation though the water makes the smoke much "cleaner"than conventional cigarette smoking. However, a recent analysis of toxicant yields, as assessed by a smoking machine, reported that the content of nicotine, CO, polyaromatic hydrocarbons, volatiles aldehydes and "tar"inhaled during 1-2 hours of water-pipe smoking were equivalent to an impressive amount of 100 to 200 cigarettes! **Case Report.** We report on a young student and American Football player complaining of reduced concentration capacity and declined physical performance associated with polyglobulia. In his first visit to our outpatient clinic the patient denied symptoms related to myeloproliferative neoplasms (itching, erythromelalgia, and constitutional symptoms) or cigarette smoking. His personal history was uneventful and the clinical assessment showed an athletic, slightly overweight and hypertensive patient with a noticeable facial plethora but without lymphadenopathies or organomegalies. The peripheral blood smear showed an isolated increase of

the hemoglobin concentration to 201 g/l (hematocrite 55%) with suppressed serum erythropoietin. The molecular analyses for JAK2 Exon 12 and 14 mutations as well as for the BCR-ABL fusion transcript were negative. In his second visit, the patient disclosed his daily Shisha-smoking habit that was usually lasting for one to two hours a day and occasionally accompanied by nausea, dizziness and one even a syncopal episode. He explained having started his excessive Shisha-smoking after break-down of the relation-ship with his girl-friend six month ago. Moreover, we learned from the patient that Shisha-smoking was trendy in his American Football player peer-group. The blood gas analysis revealed a hemoglobin carbon monoxide (COHb) of 6. 1%, in line with an accidental CO intoxication induced by excessive water-pipe smoking. The patient stopped immediately his habit and the blood-values returned to normal ranges within a month. **Summary and Conclusions.** To the best of our knowledge, this is the first case-report of secondary polyglobulia caused by accidental CO intoxication related to excessive water-pipe smoking. Due to the increasing popularity of water-pipe smoking and the anticipated health risks, we urge the policy makers to spread information on the relevant toxicities of water-pipe smoking by means of the currently running tobacco prevention programs.

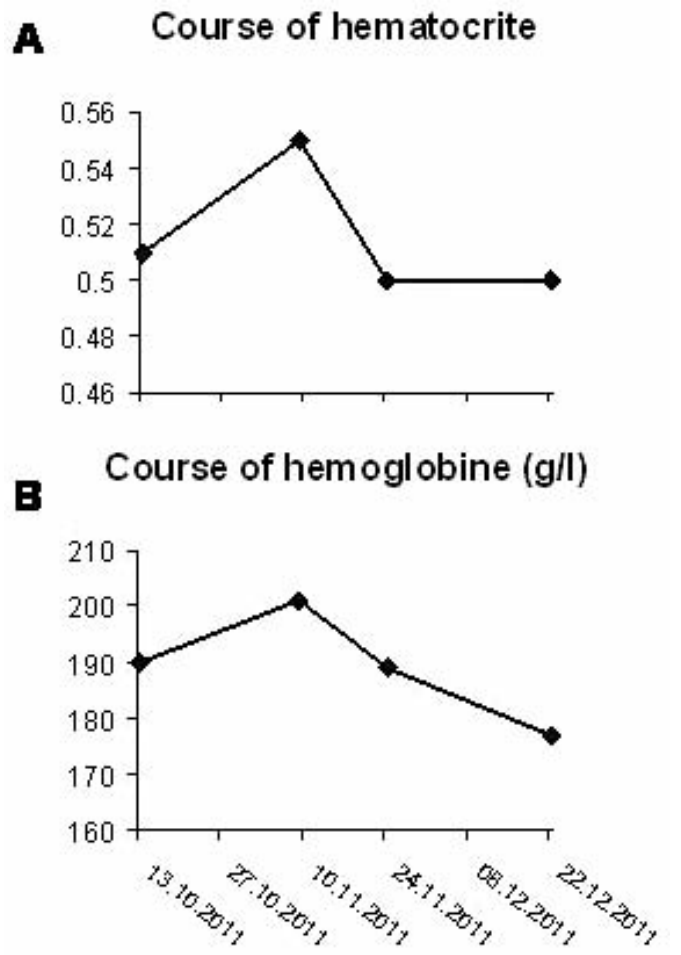

Figure 1. Course of hematocrite and hemoglobin after stopping water-pipe smoking on 10. 11. 2011.

1821

CLINICAL AND LABORATORY FEATURES OF 26 PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA IN SAO PAULO -BRAZIL

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disease, due to a clonal somatic mutation in the hematopoietic progenitor cells that become susceptible to complement-mediated hemolysis. The clinical course is extremely variable, characterized by hemolytic anemia, cytopenias and thrombotic risk. To date, there are few data on clinical features of PNH in Brazilian patients. **Aims.** To characterize clinical and laboratory features of patients with PNH in two centers in Sao Paulo, Brazil. **Methods.** Twenty six patients were evaluated. Informed consent was obtained by personal interview with all patients that participated of this study. Median age was 35 years (range: 22-81year), of which 16 female (62%). Diagnosis and clone size determination were performed by multiparametric flow cytometry. Median follow up time was 45 months (range: 4-310). **Results.** Most patients had classical PNH (19 cases, 73%), followed by PNH in the setting of other bone marrow failure syndrome (5 patients, 19%) and subclinical PNH (2 patients, 8%). Hemoglobinuria was the most frequent symptom reported by 80% of patients, followed by fatigue (69%) abdominal pain (42%) headache (30%) and dysphagia (26%). Erectile dysfunction was reported by 30% of male patients. Ten patients (38%) had a history of venous thromboembolism: deep vein thrombosis/pulmonary embolism in 4 cases, intra-abdominal veins in 3 cases (Budd-Chiari, mesenteric, portal e renal vein), and 2 cases in atypical sites (retinal and submammarian). Two patients had arterial thrombosis: two cases of ischemic stroke and one case transient ischemic attack. Currently, 4 patients are receiving anticoagulation: 2 for primary prophylaxis. Median between the start of symptoms and the diagnosis was 11 months (range: 2-133 months). At diagnosis, median hemoglobin level was 10. 5 g/dL (range: 4. 3-13. 5 g/dL), WBC 4. $7x10^9$ (1. 2-14. $2x10^9$), platelets $125x10^9$ (10-$829x10^9$), LDH 1,130 U/L (278-3989U/L), and creatinine 0. 9 mg/dL (0,54-1,51 mg/dL). Flow cytometry data was available for 22 patients at diagnosis: median erythrocyte PNH III (CD59 negative) clone was 26% (range: 0. 5-67%), median PNH II (CD59 partial deficiency) clone 28% (5-66%), monocyte CD14 negative clone 83% (2-99. 9%) and granulocyte CD24 negative 75% (2-99. 6%). Most patients (73%) had a granulocyte clone larger than 50% and 5 patients (19%) larger than 90%. Immunosuppressive therapy had been used by 17 patients (65%): 9 patients (35%) had used steroids, 3 (12%) steroids and cyclosporin, and 5 (19%) steroids, cyclosporin, and antithymocytic or antilymphocytic globulin. Twenty patients (77%) had received transfusions. Currently, 16 patients (62%) are on treatment with eculizumab. **Summary and Conclusions.** We observed a large proportion of patients presenting hemoglobinuria, with history of thrombosis and large clones. These features might be attributable to a bias selection of more severe cases referred to tertiary centers in Brazil. Besides, there was a large number of patients receiving steroids and immunosuppressive therapy, although most patients had classic PNH, emphasizing the need for national standardization of PNH management.

1822

ERYTHROPOIESIS RESPONSE MARKERS TO HYDROXYUREA THERAPY IN PATIENTS WITH SICKLE CELL DISEASE

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Introduction. The use of hydroxyurea (HU) in sickle cell disease (SCD) patients, either with homozygous sickle cell anaemia or with double heterozygosity of sickle cell anaemia and beta thalassemia (HbS/beta-thalassaemia) reduced the incidence of painful crises, transfusion requirements, hospital admissions and acute chest syndrome. Previous studies have suggested that despite the myelosuppression on haematopoiesis, HU shows a beneficial effect on SCD erythropoiesis. Reticulocyte haemoglobin equivalent (RET-He) is considered to be an actual indicator reflecting functional iron availability for bone marrow erythropoiesis, and could be used as an early marker of erythropoiesis during HU treatment. **Aims.** The purpose of this study was to further examine erythropoiesis activity in patients with HbS/ beta-thalassaemia using the reticulocyte haemoglobin equivalent (RET-He) parameter. **Methods.** Fifty seven iron-sufficient patients with HbS/beta-thalassaemia were included in the study, based on transferrin saturation values (TSAT) (>20% criterion for inclusion). TSAT was calculated as follows: serum iron (μg/dl) x 100 divided by total iron-binding protein (μg/dl). Haematological parameters RET-He, mean corpuscular volume (MCV), haemoglobin (Hb), red blood cell count (RBC), absolute reticulocyte number (RET#), and platelet count (PLT) were determined in automated analyzer SYSMEX XE-5000 (Sysmex Corporation, Kobe, Japan). Fetal haemoglobin (HbF), and sickle haemoglobin (HbS) were quantified using high performance liquid chromatography (HPLC), using Variant Bio-Rad HPLC (Bio-Rad Laboratories, Hercules, CA, USA). **Results.** The patients were divided in two groups according to HU treatment. The first group consisted of 37 patients treated with HU, and the

second group of 20 non-HU treated patients. In the first group the mean values ± standard deviation of the parameter tested were for RET-He: 25,7±4,1pg, MCV:83,5±8,2fL, Hb:9,8±1,5g/dl, RBC:3,5±0,7M/µl, RET#:19,7±10,8x10^4/µl, PLT:267±151. 3K/µl, HbS:67,1±7,8%, HbF:21,1±8,3%. In the second group were respectively 21±2. 6pg, 64,5±5,5fL, 8,9±0,9g/dl, 4,2±0,69M/µl, 26,2±13,1x10^4/µl, 366,6±189,6K/µl, 75,2±6,8%, 10,3±6,4%. Significantly increased values with p<0. 05 revealed for RET-He, HbF, MCV, and Hb in the patients treated with HU compared to the non-HU treated, whereas the HbS, red blood cell count, absolute reticulocyte number, and platelet count were found significantly decreased (p<0. 05). **Conclusions.** The mean values for reticulocyte count in both groups were increased in response to tissue hypoxia and haemolysis, Although, the significant decrease of reticulocyte count during hydroxyurea therapy could be suggestive of myelosuppression or decrease in haemolysis as a result of hydroxyurea therapy, the increase of Ret-he (p<0. 05) in the HU group is supportive of bone marrow erythropoiesis activation. Among the changes that found in the haematological parameters examined, due to administration of HU, the RET-He could be added, in order to be used as an early marker of response to the HU-therapy in patients with HbS/beta-thalassaemia. Further investigation is needed in order to confirm this finding.

1823

THALASSEMIA INTERMEDIA AND CONGENITAL DYSERYTHROPOIETIC ANEMIA (CDA) LEAD TO IRON OVERLOAD IN THE ABSENCE OF REPEATED BLOOD TRANSFUSIONS

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Background. Some patients with chronic anemia like β-thalassemia intermedia (TI) or congenital dyserythropoietic anemia (CDA) rarely or never need transfusion therapy. However, irrespective of their transfusion status, they develop substantial iron overload. The enhanced erythropoiesis results in hepcidin suppression, leading to increased iron absorption in the gut and increased release of recycled iron from the reticulo-endothelial system (Origa et al, 2007). Body iron levels can be determined by measurement of serum ferritin and of liver iron concentration (LIC). It has been shown in several studies of patients with TI that the relation between serum ferritin and liver iron is different from that in ß-thalassemia major (Origa et al, 2007; Pakbaz et al, 2007; Taher et al, 2008). **Aims.** We aim to determine the body iron load of non-transfused TI or CDA patients and to compare LIC and serum ferritin as markers for iron overload. **Methods.** We measured LIC and ferritin in 15 patients (5 CDA, 10 TI, median age 27 years, range 10-59 years) who were never or minimally transfused. LIC was measured by SQUID biomagnetic liver susceptometry. **Results.** The median LIC in our cohort was 1900 µg Fe/g-liver (in vivo wet weight), range 200 - 4900, and median ferritin 677 µg/l, range 101 - 2119. The correlation of age with LIC was more significant (r = 0. 72, p=0. 003) than with serum ferritin (r=0. 59, p=0. 02), while the correlation of LIC with ferritin was similarly significant (r=0. 56 (p= 0. 03). When judged by serum ferritin only, 11 patients would not have been deemed to require iron chelation therapy (SF < 1000 µg/l). However, 3 of them had LIC levels between 1000 and 2000 µg Fe/g-liver, and a further 4 patients had LIC levels > 2000 µg Fe/g-liver, which is a clear indicator of severe liver siderosis. **Conclusions.** More than 60% of patients with only moderately elevated ferritin (< 1000 µg/l) show clear liver siderosis as measured by LIC. Thus, serum ferritin systematically underestimates body iron load. Patients with non-transfused TI or CDA need direct assessment of liver iron concentration to guide therapy. The study is ongoing and we will present further analysis of a larger cohort of patients.

1824

SPLENIC FEATURES IN SICKLE CELL ANEMIA AND IN SICKLE BETA-THALASSEMIA PATIENTS

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Background. Sickle Cell Disease (SCD) is one of the most common severe monogenic inherited disorder worldwide. The haemoglobin polymerization, leading to erythrocyte rigidity and vaso-occlusion, is central to the pathophysiology, as well as chronic anaemia, haemolysis, and vasculopathy are crucial for the clinical outcome. Sickle Cell Anemia (SCA) and Sickle beta-Thalassemia (T-SCD) are multiorgan diseases with similar findings associated with episodes of acute crisis and progressive organ damage. **Aims**. This is a retrospective study aimed to compare the clinical and splenic findings of SCA patients with T-SCD patients cared at Hereditary Anemia Centre, Foundation IRCCS “Ca’ Granda”Ospedale Maggiore Policlinico, in Milan, Italy. **Methods**. Mutation analysis of the beta globin gene was performed by direct DNA sequencing by the ABI Prism 310 genetic analyzer. Clinical and haematological parameters were evaluated by routine tests and physical examination according to guidelines for SCD follow-up. **Results**. Sixteen SCA and 43 T-SCD patients were studied. The beta-mutations detected in T-SCD were severe (beta°) in 86%, moderate (beta$^+$) in 11. 5% and mild (beta^{++}) in 2. 5% of patients respectively. The mean age was 38±10 years, and the mean follow-up is 20±6 years. In SCA group 5/16 (31. 2%) and in T-SCD group 17/43 (39. 5%) were male. In SCA HbF mean levels was 7. 3±5. 4% and in T-SCD 10. 1±7. 1%, while Hb total was around 95 g/l, similar in both groups. Comparing SCA and T-SCD patients, there were not statistically significant differences in the prevalence of clinical manifestations, including stroke, leg ulcers, priapism, bone pain crisis, except for spleen features. Splenectomy was performed in 2/16 (12. 5%) SCA patients vs 21/43 (48. 8%) T-SCD (*p*-value < 0. 001). Splenomegaly was absent in SCA patients, while was detected in 11/22 (50%) T-SCD. Fourteen/16 (87. 5%) SCA patients had functional asplenia. Splenic infarctions were present only in T-SCD patients (7/22; 31. 8%), in particular in 5/11 (45%) of patients with splenomegaly and in 2/11 (18%) of patients with normal spleen size (*p* value < 0. 001). No spleen lesions were detected in SCA patients. All the patients were only occasionally transfused; however T-SCD patients were slightly more transfused than SCA patients during life. In all the T-SCD patients in which the first blood transfusion was performed in childhood, we observed normal spleen sizes. Eighteen/43 (41. 8%) T-SCD patients and 6/16 (37. 5%) SCA were treated with hydroxyurea started in the adult life. **Conclusions**. These data suggest that T-SCD patients, particularly those with severe beta mutations have similar clinical course than SCA patients. T-SCD patients develop splenomegaly that correlate with the age of first transfusion: in particular when the first blood transfusion occurs in the childhood, the development of splenomegaly and spleen infarct seem to be prevented.

1825

THE EFFECTS OF ORAL FERROUS GLUCONATE SUPPLEMENTATION ON HEALTH-RELATED QUALITY OF LIFE IN NON-CANCER PATIENTS WITH IRON DEFICIENCY ANEMIA

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Background. It is known that iron deficiency is characterized by a variety of adverse effects on the physical and intellectual spheres of human. However, data on the effects of mild iron deficiency anemia (IDA) and its treatment on quality of life (QoL) are limited. **Aims.** To examine whether changes in hemoglobin (Hb), serum ferritin and iron levels were associated with changes in QoL in response to oral iron supplementation in a patients with IDA. **Methods.** Forty-four patients with anemia diagnosed as Hb level ≤ 12. 0 g/dl were enrolled in this study. In addition to the direct presence of anemia, iron deficiency confirmed the changes at least one of the following biochemical parameters: (1) serum iron levels < 6. 6 mmol/l; (2) unsaturated iron binding capacity > 64 mmol/l; (3) concentration of transferrin > 3. 6 g/l; (4) transferrin saturation < 18. 4% and/or (5) serum ferritin < 5 ng/ml. All patients were treated with oral ferrous gluconate (Tot’hema®, Laboratoire Innotech International, France) at the dose of 50 mg twice daily for at least 3 months. Assessment of patients, including adverse events, hematological and biochemical blood tests, and QoL was performed before treatment (point 0) and at 1 and 3. 5 months. QoL was assessed using a general questionnaire SF-36 (version 2. 0). Analysis of the QoL was carried out for 43 (98%) patients, and 1 patient with later diagnosed colon cancer was removed. **Results.** The median age was 42 years (range 20-84). All patients were female. The most common causes of IDA were gynecologic - 18 (41%) and gastrointestinal tract hemorrhages - 5 (11%). Normalization of Hb levels (15. 0 ±3. 4 g/dl vs. baseline 10. 2 ±0. 3 g/dl, P<0. 05) was confirmed at 1 month of therapy in all patients. Increased levels of serum iron (15. 8 ±1. 6 mkmol/l vs. baseline 6. 9 ±0. 8 mkmol/l, P<0. 05) and ferritin (14. 3 ±1. 6 g/dl vs. baseline 6. 0 ± 0. 7 g/dl, P<0. 05) were also confirmed. After 9 months of a normal Hb levels 12. 7 ±0. 4 g/dL (normal values 12. 3-15. 3) and serum ferritin - 16. 9 ±3. 7 ng/ml (normal values 5-148) was maintained. Improving QoL was documented after 3. 5 month of treatment on all physical and mental scales of SF-36. The only factor in the improvement of QoL was an increase in Hb levels (Figure 1). The graph shows the ratio between the average values of QoL after therapy (3. 5 months) compared with baseline (0 months) for patients with an increase in Hb levels <20% (n=14) and ≥ 20% (n=30). The increase in Hb levels was accompanied by significant improvements in ‘Mental Component of Health - MH» (1. 34 ±0. 17 vs. 0. 87 ±0. 14, P<0. 05) rather than ‘Physical Component of Health - PH» (1. 10 ±0. 08 vs. 0. 96 ±0. 07, P>0. 05). Increase in lev-

els of serum iron and ferritin as well as the adverse events associated with therapy had no effect on QoL. **Conclusions.** Only an increase in Hb levels, but an increase of serum iron and ferritin levels impact on QoL in patients with IDA treated with oral iron.

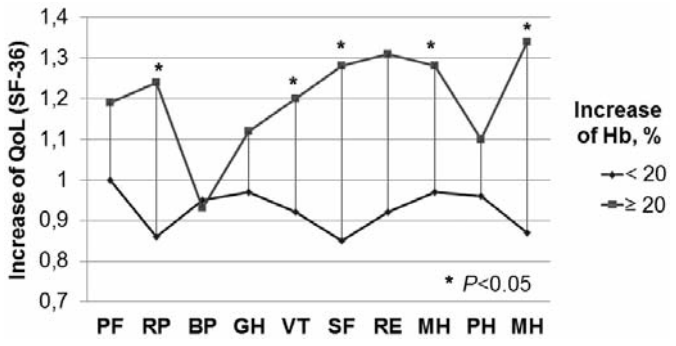

Figure 1. The increase in hemoglobin levels correlated with improved QoL.

CONGENITAL METHEMOGLOBINEMIA CAUSED BY HEMOGLOBIN M SASKATOON HETEROZIGOTE (?-63CAT ? TAT, HIS ? TYR): DISCREPANCY ON DIAGNOSIS

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Background. Hereditary methemoglobinemia caused by hemoglobin M (Hb-M), is a rare disorder, due to a structural defect in the alpha or beta globin chains of the hemoglobin, producing a permanent oxidation of heme iron to the ferric state. The ferric hemes of methemoglobin are unable to bind oxygen, with an impared oxygen delivery to the tissues and lifelong cyanosis. **Case Report.** A 22-month-old female, born by cesarean section because of fetal distress, Apgar score 3 at first minute and 9 at five minutes. History of chronic cyanosis since birth, without associated jaundice. No relevant family history, or trigger medication administration. Examination: Moderate failure to thrive with adequate statural development, good performance status, adequate level of consciousness. Central cyanosis and on nail beds; cardiac or pulmonary causes of cianosis were excluded; soft and depresible abdomen, no palpable organomegaly. Oxygen saturation by pulse oximetry: 97%. Functional and structural assessment of the cardiopulmonary system within normal limits. Laboratory: Macroscospically, the blood was dark brown chocolate color, and not modified after oxygenation. Complete blood count: Hbg: 11 g/dL, Hct: 34 % MCV: 86,8 fl. Leukocytes 10,3 x 10^9/L with normal diferential count, Platelets: 292 x 10^9/L, HbF: 0,05 %. Slight hemolysis component, with good reticulocyte response. Parametres of ferric metabolism, seric levels of folic acid and vitamin B_{12} were normal. Hemoglobinopathies studies:Hb electrophoresis on cellulose acetate (pH 8,6): bands of anomalous migration were not observed. The apparently normal fractions presented a chocolate-coloured dye. HPLC: an anomalous peak of 10. 3% was detected in window C at 143 seconds, which suggested the presence of an anomalous hemoglobin. Spectrometry: an anomalous slope in the curve of Hb was appreciable, with a peak of extinction at 602 nm. Reaction was not observed with the addition of potassium cyanide and with the subsequent addition of ferricyanure, peaks of extinction were observed at 490 nm and 602 nm. Eritrocitary enzymes: Glucose 6-phosphate dehydrogenase: 9,5 IU/gHb (N. V: 7-12), Pyruvate kinase: 43,3 IU/gHb (N. V: 9-15). Unstable hemoglobins test: Isopropanol positive (++), Heat test: negative. $P_{50}O_2$: 39 mmHg (N. V: 26,8mmHg): decreased oxygen affinity. Molecular biology: the analysis of the β globin showed a C→ T substitution at codon 63. This leads to a replacement of histidine (CAT) by tyrosine (TAT) at the β 63 (E7), Hb-M Saskatoon. **Diagnosis.** Congenital methemoglobinemia by Hb-M Saskatoon heterozigote (β-63CAT → TAT, His → Tyr). A clinical/laboratorial discrepancy exists, owing to cyanosis since birth which suggests an alteration of the alpha globin chains, unlike the molecular biology study was compatible with an alteration of beta globin chains. **Conclusions.** Chronic cyanosis secondary to HbM is usually the only one sign of presentation. In some patients, the anemia with mild hemolytic component is developed, according to the type Hb-M, which is well tolerated due to the decreased affinity by the oxygen, characteristic of this alteration. It is advisable to perform a familiar study and genetic advice.

1827

ASSESSMENT OF THE EFFICACY AND SAFETY OF INTRAVENOUS IRON SUCROSE IN THE TREATMENT OF IRON DEFICIENCY ANEMIA (IDA)

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Background. Iron deficiency is the most common form of anemia worldwide. It is an important health issue in developingcountries and a frequent cause of anemia even inWestern Europe. The treatment for iron deficiency anemia consists of replacement iron either orally or parenterally. Patients with iron deficiency anemia (IDA) who need intravenous iron are normally treated with iron dextran. One of the major side effects of iron dextran is severe (sometimes fatal) anaphylactic reaction, which can develop in about 1% of patients. On the other hand, iron sucrose another intravenous iron preparation that has improved safety and ease of administration. **Aims.** The aim was to evaluate the therapeutic effectiveness and safety of intravenous iron sucrose therapy in adult patient with IDA. **Methods.** A prospective study conducted at the national center of hematology/Almustansiriya university, Baghdad. it included 168 patients diagnosed as iron deficiency anemia from May 2009 to october 2011. All patient gave their written informed consent. complete blood count, reticulocyte count, serum iron, total iron-binding capacity . and serum ferritin done for all patients. Total dose of Iron sucrose Venofer® was calculated and administered in a weekly successive session in which total dose in each session not exceed 500 mg elemental iron in 500 ml 0. 9% normal saline infusion over 3-4 hour . Charts of patients were reviewed and key labratory tests were done before the start of treatment, 2 weeks and 1 month after starting the treatment. Response was defined as an increase in the hemoglobin level of 2 g/dl or greater after starting the treatment. At the same time, the reason for starting the IV iron therapy and the toxicity associated with treatment was evaluated. The paired t test was used to assess hemoglobin response. **Results.** Median age of the patients was 28 years range from 16 to 66 years. Ten patients (5. 9%) were male while 158 (94. 1%) were female. Before treatment, The mean hemoglobin, MCV, serum iron and serum ferritin values were 8. 1 g/dl, 65. 42 fl, 3. 67 mmol/l and 9. 30 ng/ml pre-infusion and 11. 4 g/dl, 78. 95 fl,15. 20 mmol/l and 66. 78 ng/ml after 1 month of treatment ($p < 0.001$), respectively mild reaction was noted in 4 patient only in form of numbness and itching at the infusion site in 4 patients (all of them received 500 mg iron sucrose in one session patients. The therapy was generally well-tolerated and major side effects were observed The mean duration to achieve the Hb level of 11 gm/dl or more was 4 weeks. **Conclusions.** Our data showed that intravenous iron sucrose Venofer®, which is currently not the standard of care for treatment of IDA in non-dialysis-dependent patients has very good response. It is well tolerated and has no major side effects. **Disclosures**. Off Label Use: iron sucrose Venofer® for iron deficiency anemia in non-dialysis dependent patients.

Table 1. Comparison of the results of the main laboratory variables used at the beginning and after 1 month of treatment with IV iron sucrose (IS) administred to 168 patients with iron deficiency anemia.

variable	Before IS Infusion Mean (SD) min-max	After IS Infusion Mean (SD) min-max	P value
Hb level (g/dl)	8.1 (1.30) 5,40-10,60	11.4 (1.36) 9,0-14,80	<0.001
S. Iron level (Mmol/L)	3.67 (11.91) 0,70-59,00	15.20 (24.97) 5,00-115,00	<0.001
TS (%)	9.23 (2.49) 6.00 to 19.00	23.12 (15.06) 11.00 to 48.00	<0.001
MCV (fl)	65.42 (6.96) 53,00-84,00	78.95 (4.97) 70,40-93,00	<0.01
Ferritin level (ng/dl)	9.30 (4.02) 1,00-14,80	66.79 (82.39) 5,30-383,00	<0.001

SD = standard deviation, Min = minimum, Max =maximum,
MCV = mean corpuscular volume; TS = transferrin saturation

1828

COMPOUND HETEROZYGOSITY OF THE -101 C?T SUBSTITUTION OF THE ?-GLOBIN PROMOTER WITH OTHER THALASSAEMIA MUTATION. MOLECULAR, HAEMATOLOGICAL AND CLINICAL PHENOTYPE IN FIVE CASES

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The β-Thalassaemias constitute an heterogeneous group of disorders with a reduction of β-chain synthesis. The interaction of silent with classic β-Thalassaemia mutations results in the clinical phenotype of Thalassaemia Intermediate. Among the silent β-Thalassaemias the -101 C→T substitution from the Cap site within the distal CACCC box is considered the most common silent mutation in the Greek population. Heterozygotes of the -101 C→T substitution have normal or borderline hematological indices. Compound heterozygotes with mild non-transfusion-dependent Thalassaemia Intermediate of the -101 C→T mutation with other β-Thalassaemia mutations have been identified due to the progress in molecular diagnosis. We report the molecular, haematological and clinical phenotype of 5 cases of compound heterozygotes of -101 C→T mutation in combination with IVSII-745, β-87, IVSI-110, and -101 C→T. The hematologic analyzer, Coulter ONYX, was used to determine red cell indices (RBC, Hb, Ht, MCV, MCH, RDW). Bio-Rad Variant Haemoglobin Testing System (Bio-Rad Labs., Hercules, CA), the 'ß Thalassaemia Short' program, an automated Cation Exchange - High Performance Liquid Chromatography (CE-HPLC) instrument was used for the quantification of HbA, F, A2, S, C, and other haemoglobins. Haemoglobin A2 levels were also quantified by column microchromatography (Hb A2 Helena Lab.). Serum ferritin levels were measured by micro-Elisa technique (Abbot Lab.). Of the five patients, one was diagnosed during childhood, one in infancy and three in adult life. All patients have satisfactory growth and development. Only one patient with the -101/IVSI-110 combination had mild splenomegaly and erythroblasts presence in the peripheral blood smear but was never transfused. In three of the cases Hb A2 levels were increased. The homozygote of the-101 C→T /-101 C→T had a very mild clinical phenotype as a heterozygote of β-Thalassaemia. The only 45 days baby is followed up. The genotype and phenotype expression correlation gives important information for the genetic counseling of couples at risk and helps to predict the prognosis in each patient.

Table 1.

mutation	101/IVSII 745	-101/-101	-101/β-87	-101/IVSI-110	-101/IVSII 745
Age (years)	35	35	45 days	53	8
Hb(g/dl)	9,1	12,5	9,4	10,2	10,7
Ht(%)	29,1	37	27,3	32	32,5
MCV(fl)	60,4	4,980	85	62	54,3
MCH(pg)	18,9	27,1	24,6	18,9	17,9
RDW(%)	16,8	13,4	21,4	25	25,4
RBC	4,82	4,62	3,19	5,16	6,00
HbA2(%)	7,4	4,9	1,1	7,3	7,2
HbF(%)	2,8	0,8	85,9	10,1	2,3
Ferritin(µg/l)	17	35	-	99	81

1829

RETROSPECTIVE ANALYSIS OF PATIENTS WITH VITAMIN B12 DEFICIENCY WHO WERE FOLLOWED AND TREATED IN PEDIATRIC HEMATOLOGY DEPARTMENT

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Aims. In this study, the analysis of the frequency, demographic features, causes of deficiency, other diseases, the clinical and laboratory features of the patients with vitamin B12 deficiency and the evaluation of the outcome of treatment are aimed. **Methods.** The records of the 145 patients with vitamin B12 deficiency aged 0-18 years (0-215 months) followed between January 2006 and December 2010 in Ondokuz Mayis University Faculty of medicine, department of Pediatric Hematology analyzed retrospectively. **Results.** The frequency of vitamin B_{12} deficiency was observed at least 2. 8 %. 82 (57%) patients were female, 63 (43%) patients were male and the frequency was higher in females and between 0-2 years and 12-17 years (in adolescents). The mean of weaning the of infants was 9,6±3,81 months and it delayed. 77% of the mothers had vitamin B_{12} deficiency. There was no correlation between the vitamin B12 levels of infants and their mothers. The most frequent symptoms on admission of patients were pallor (43%), fatigue (40%), anorexia (32%), insufficient weight gain(23%), vomiting (18%). 43% of the patients had pallor on skin and mucosa, 29% had growth failure, 18% had development delay, 15% had apathy-irritability. The mean vitamin B_{12} level was 145±37,3 pg/ml, the mean hemoglobin level was 9,82±2,96 g/dl, the mean MCV was 81,8±15,46 fl. The 57% of patients had anemia, 16% had neutropenia, 17% had bicytopenia, 9% had thrombocytopenia, 4% had pancytopenia, 30% had macrocytosis. The 57% of patients had iron deficiency, 5% had parasite infestation, 5% had HP infection, 3% had celiac disease, 2% had FMF and history of colchicum use. The 97% cause of vitamin B12 deficiency was nutritional. Even though all patients had vitamin B12 deficiency, only 44% of them had higher homocystein levels. In treatment group, 51% of patients received cyanocobalamine oral route, 49% received IM. The IM treatment was more effective than oral route. The four of 116 patients (3. 5%) had complication after treatment. **Conclusions.** This study showed that vitamin B12 deficiency is frequent in children in samsun and surrounding cities. The disease becomes widespread first two years and between 12-17 years. This indicates that these ages are at risk. To prevent vitamin B12 deficiency in infants, its level must be evaluated in pregnant and mothers of infants and foods of animal origin must be suggested. Vitamin B_{12} deficiency must be evaluated as a public health problem and the prevalence studies must performed regional and in country and new solutions must produced.

1830

THE SUCCESSFUL TREATMENT OF RESISTANT FORM OF AUTOIMMUNE HEMOLYTIC ANEMIA BURDENED WITH VIRAL HEPATITIS B AND C WITH RITUXIMAB (MABTHERA)

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Autoimmune hemolytic anemia (AIHA) - is characterized by the production of autoantibodies to red blood cell (Rbc) surface antigens with destruction of red blood cells by complement and reticuloendothelial system. The scientists have accumulated much experience in treatment of refractory form of AIHA with Rituximab (Mabthera) during last few years. But the patients with the viral hepatitis are restricted in treatment with this medicine. **Methods and Results.** We observed 20 patients (age 21-67 y) with resistant form AIHA (19 - AIHA, 1- Evans syndrome), with duration time 6-72 month. They have been resistant for steroid therapy, splenectomy, immunosuppressive drugs (azatioprine, cyclophosphamide). In hemolytic period (Hb 35-67 g/l) they have been treated with Rituximab (Mabthera) 375 mg/m^2, 2 weekly doses. This short treatment was enough as B lymphocytes became undetectable in all patients (CD19-0%, CD20-0%). Four of the 20 patients suffering also with resistant form AIHA exibited hepatitis B or C (n=2 and n=2). The patient with Evans syndrome was diagnosed as manifesting hepatitis B after 2 weeks therapy with Mabthera (he was transfused by the Rbc for 2 months). He had an acquired hepatitis with skin jaundice. The Hb level was 98 g/l (after steroid therapy), tr- 18x10^9, ret-80%$_0$, bilirubin rise, was 4 times of norm, ALT, AST - 6 of norm, LDG - 5 of norm, and it was found that virus level has raised in 3 of normal value. All parameters were normalized after 2 weeks treatment with lamivudin (100mg). Treatment with lamivudin has been lasting for 2 years. After 6 month of therapy evaluated reduction of viral load in 2 times that of the normal and were not determined after 1 year of treatment. Remission of AIHA was achieved in a 1 month after Mabthera therapy applied. The second course of Mabthera was carried out during of lamivudin treatment for a year, in the interval time of the hemolysis appearing again. The virus activation was not detectable. The second patient with chronicle hepatitis B received the Mabthera therapy when severe hemolysis developed (Hb 35 g/l, ret-220%$_0$). This patient had hard complications - vein tromboses of legs, pneumonia and sepsis (Gr+). He has been in artificial lung ventilation for 29 days. During this period replication of the virus hepatitis B was not observed and new infectious complications were not revealed. Remission of AIHA was achieved in 1,5 months. Other 2 patients with hepatitis C didn't demonstrate hepatitis C virus replicationafter treat-

ment with Mabthera, remission period in patients with AIHA was reached in 9,12,15,24 months. **Conclusions.** The patients with resistant form of AIHA with hepatitis B, could receive the treatment of Mabthera in combination with antiviral drug - lamivudin. For the patients with hepatitis C - in periods without exhibition of viral hepatitis activity, a constant laboratory control of hepatitis C marker is recomended.

1831

TRANSFUSION-DEPENDENT CLINICAL PHENOTYPE IN PATIENTS WITH COMBINED HETEROZYGOSITY FOR A SILENT (+1480 C>G) AND CLASSIC BETA-THALASSEMIA MUTATIONS: REPORT OF 4 CASES

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Background. The beta-thalassemias are a group of inherited disorders of red blood cells resulting from reduced or absent production of the beta-globin of hemoglobin and characterized by considerable molecular and clinical heterogeneity. Carrier-ship of a silent beta-thalassemia mutation becomes clinically apparent when co-inherited with a typical beta-thalassemia mutation. The clinical phenotype of the above combination is usually that of mild, non transfusion-dependant thalassemia intermedia. **Aims.** To report the cases of 4 patients with double heterozygosity for a silent (+1480 C>G) and typical beta-thalassemia mutations, resulting to a transfusion-dependent clinical phenotype. **Patients.** Patients involved are 4 boys aged 8 to 9 years old (mean age: 8. 7 ± 0. 5), diagnosed with thalassemia at the age of 2 to 6. 5 years. Main hematological parameters were as follows: Hb 8. 25 ± 0. 24 g/dl (7. 3 - 8. 8), MCV 60. 6 ± 5. 3 fl (57-67. 5), MCH 16. 5 ± 3. 8 pg (11 - 19. 8), HbF 11. 6 ± 7. 4% (2. 6 - 29). The first patient was a double heterozygote for the +1480 (C>G) and the CD39 mutation, while the second patient was a double heterozygote for the +1480 (C>G) and the IVS1-1 mutation. Due to facial and skeletal changes, both patients were put on a regular transfusion program from the ages of 7 and 6 years, respectively. The third patient was doubly heterozygous for the IVS1-110 and +1480(C>G) mutations. Due to hypersplenism he was put on an early transfusion program. Finally, the fourth patient was doubly heterozygous for the IVS1-110 and +1480(C>G) mutations. He had incomplete follow-up until the age of 9, when he was referred to our center and immediately put on regular transfusions due to splenomegaly and concomitant hypersplenism. It must be noted that an excess of alpha-globin genes was not detected in any of the patients. **Conclusions.** Although combined heterozygosity for a silent and a typical beta-thalassemia mutation usually results to a non-transfusion dependent thalassemia phenotype, there are severe clinical exceptions. When managing with such patients, evaluation of both genotype and clinical parameters is imperative. Genetic guidance remains difficult in such cases.

1832

DEFERASIROX EFFICACY IS INDIPENDENT BY IMPROVEMENT OF IRON OVERLOAD BIOCHEMICAL MARKERS IN TRANSFUSION DEPENDENT MYELODYSPLASTIC PATIENTS

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Iron chelating therapy (ICT) has become an important therapeutical opportunity in chronic transfusion dependent anemia as the one most myelodysplastic (MDS) patients suffer from, since the availability of oral chelating drugs as Deferasirox. ICT allows these patients to be prevented by iron overload organ damage (heart, liver) which has been demonstrated to negatively affect their life expectancy. There's evidence that some transfusion dependent chronic anemic patients who are on ICT show an improvement in hematological parameters and that a few of them experience a reduction in transfusional need (probably related to the reduction of: oxidative stress in erythroid bone marrow precursors, ROS generation, lipid peroxidation and free iron levels. It is also thought that iron chelating agents can let iron become available for erythropoiesis). That's what we have seen in eleven transfusion dependent MDS patients(5 RA, 2 RARS-T, 1 CMMoL, 1 RARS, 1 RCMD, 1 RAEB-1)who started oral ICT (Deferasirox). We have seen an improvement in hemoglobin level and a reduction in transfusional need. This effect has appeared to be evident in most of the patients within a few months; none of them went on to other therapies that could be able to modify erythropoiesis. What seems interesting is that these positive effects appeared to be independent by significative modification of iron overload biochemical markers as the most widely used ferritin concentration. Another result we have noted has been a progressive reduction in liver enzymes concentration in patients who showed biochemical evidence of liver damage before beginning ICT. Also this effect appeared to be independent by a significative modification of ferritin levels. All these results have been noted also in those patients who had to be treated with ICT doses lower than conventional ones because of sever comorbidities they were affected by (as renal function impairment). The possible explanation to our results is that Deferasirox is able to induce(especially at lower doses as demonstrated in "in vitro"models)intracellular oxidative stress processes reduction (independent by the effects on ferritin concentration, marker of iron overload). According to this we think that ICT may have a role in the setting of transfusion dependent anemic MDS patients even if used at low doses especially if we keep in mind that most of these patients are old and have comorbidities that can prevent them by using conventional doses.

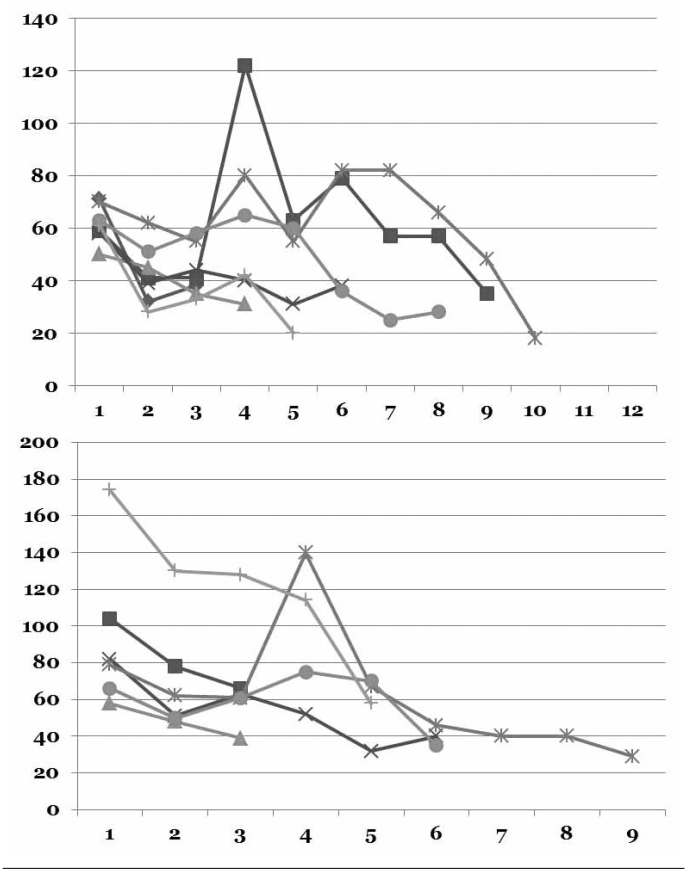

Figure 1.

1833

PATTERNS OF THERAPY AND ETIOLOGY PROFILE OF SEVERE IRON DEFICIENCY ANEMIA IN PEDIATRIC PATIENTS: 10 YEARS EXPERIENCE

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Background. Management of severe iron deficiency anemia in hospital setting ranges from oral iron therapy to red cell transfusion. Our study assessed patterns of therapy and their outcome as well as aetiology profile in children admitted with severe iron deficiency anemia at the Royal Children's Hospital in Melbourne, Australia. **Aims.** Despite the fact that iron deficiency is recognized as a chronic condition, a significant number of children with severe iron deficiency continue to receive red cell transfusion. The aim of this study is to determine therapy patterns and etiology profile among the children admitted with severe iron deficiency anemia. To our knowledge, this is the largest retrospective study so far addressing such a question. **Methods.** A retrospective study of children, 2months to 18 years (mean: 6. 2 year), admitted to RCH with severe iron deficiency anemia (Hb < 70 g/L) and serum ferritin < 15 ug/L) between January 1998 and December 2008. Results analysis was performed using statistics package social software (SPSS)Mann-Whitney U test was used for statistics analysis. **Results.**

67 patients identified: 31 male, 36 female, aged 2 months to 18 years (Mean: 6. 2 years). Overall poor diet was a leading cause of severe iron deficiency anemia (65. 6%) followed by GIT disorder (23. 4%). However the latter was more common in 10 - 18 years age group. Hb level ranged from 19 - 69 g/L (Mean: 48. 3 g/L) and serum ferritin was 1 - 14 ug/L (Mean: 2. 6 ug/L). No patients were compromised on admission. Red cell transfusion and oral iron were the most common initial therapies 46. 3% and 47% respectively, while iron infusion was used only in 6. 0% of cases. Hb level below 50 g/L was treated with red cell transfusion in 64. 5% of cases. In terms of etiology, GIT bleed was treated with red cell transfusion in 25. 8%. There was no significant difference in outcome across the 3 groups. However the oral iron group had a statistically significant shorter length of stay of 2. 6 days compared to red cell transfusion group of 4. 6 days (Z = - 2. 773, p < . 05). This could be due to the difference in medical complexity of the patients. **Conclusions.** Poor diet remains the main cause of iron deficiency in young children, followed by GIT disorders. Severe iron deficiency anemia is a chronic condition. In the absence of end organ hypoxia and significant difference in outcome, transfusion as the primary therapy appeared over-utilised, with exception of patients with concomitant acute blood loss.

1834

EVALUATION OF THE MICROVOLT T-WAVE ALTERNANS IN PATIENTS WITH THALASSEMIA MAJOR

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Backround. Cardiac complications due to iron overload are the primary causes of mortality in patients with Thalassemia major. Cardiac effects have been lately identified due to delayed appearance of related symptoms. Microvolt T-wave alternans (M-TWA) could be associated with ventricular arrhythmias and sudden cardiac deaths. There have been no studies about M-TWA in patients with beta thalassemia major up to now. **Aims.** Aim of this study to analyse the M-TWA test that can be used for early identification of cardiac effects in patients with thalassemia major with no cardiac symptoms. **Methods.** 40 patients aged between 12 and 44 years (mean 23. 6±7. 2), diagnosed as thalassemia major were included in this study. QTc dispersion (QTcd) was determined by calculating the difference between the greatest and the least QTc in the ECG. During the stress exercise tests, heart rates were allowed to reach 120-130/min and their M-TWA were calculated in this interval by Modified Moving Average (MMA) method, and the test was considered to have positive result regarding the values over 20 μV. Time-based heart rate changes parameters measured automatically from the 24-hour rhythm holter records (average HR, SDNN, SDANN, SDNN Index, RMSSD, pNN50) of the patients were recorded. Cardiac and liver iron measured by MRI (T2* and R2 respectively). Statistical analysis was made by using SPSS program with Mann-Whitney U test. **Results.** Mean ferritin level of the patients was found to be 1982. 1±1872. 5. QTc dispersion was over the limit value of 50 ms in 13 patients (35%) and mean QTc was 39. 9±19. 3. M-TWA was found to be positive in 15 patients (44. 1%). While a statistically significant association between the ferritin level and QTcd and M-TWA was not found, QTcd was observed to be significantly higher in patients with M-TWA positivity. While average HR was found to be significantly higher in patients whose ferritin level, one of the parameters of heart rate variability, was over 2500 μg/L compared to the patients whose ferritin level is below 2500 ng/ml, SDANN, SDNN-24, SDNN-I which are from the another group of parameters were found to be significantly lower. There was no statistically significant correlation between TWA positivity and cardiac iron load (T2* values). With these results obtained we conducted in patients with asymptomatic thalassemia major, it was seen that there has been no significant correlation between ferritin level and QTc dispersion and M-TWA, but there was a significant correlation between ferritin level and heart rate variability which is an indicator of the influence occurred in the sympathovagal system. **Conclusions.** Cardiac iron (high ferritin and low T2* values) is not sufficient in demonstrating the cardiac status of the patients. We recommend noninvasive tests; QTc dispersion, M-TWA in order to suggest cardiac complications who have no cardiac symptoms. This study supported by Istanbul University research Fund.

1835

CONCENTRATIONS OF HEMOGLOBIN AND BILIRUBIN AMONG FEMALE STUDENTS WITH ALPHA-THALASSEMIA, BETA-THALASSEMIA AND IRON DEFICIENCY ANEMIA

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Background. The comparisons of hemoglobin (Hb) and bilirubin concentrations among university students with α-thalassemia, β-thalassemia and iron deficiency anemia (IDA) have not yet being reported in Taiwan. **Aims.** The aim is to investigate whether Hb and bilirubin values are different or not among Taiwanese female students with α-thalassemia, β-thalassemia and IDA. **Methods.** A total of 1723 female university freshmen who attended a regular physical examination were enrolled in underwent diagnosis for α-thalassemia by PCR, β-thalassemia by PCR-restriction fragment length polymorphism (RFLP) and for IDA by measurements of serum iron, iron binding capacity and ferritin, respectively. The five known variations of the UDP-glucuronosyltransferase (UGT) 1A1 gene {polymorphisms at nucleotides □¬3279 (T>G), □¬53 [A(TA)$_6$TAA>A(TA)$_7$TAA], 211 (G>A), 686 (C>A) and 1091 (C>T)} were determined by PCR-RFLP. **Results.** Among the 1723 female students, 136 individuals whose mean corpuscular volume was ≤80 fL. Out of the 136 students, 109 subjects agreed to participate in further study. Finally, 26 students were diagnosed being IDA patients, 48 were heterozygous α-thalassemia carriers and 31 were heterozygous β-thalassemia carriers, respectively. Hb concentration in 13 of the 48 heterozygous α-thalassemia carriers and 28 of the 31 heterozygous β-thalassemia carriers was <12. 0 g/dL (anemia). Then hemoglobin and bilirubin values were compared for the 13 α-thalassemias, the 28 β-thalassemias and the 26 IDA students. Hb concentration was lower in the IDA students [mean (SD): 10. 0 (1. 14) g/dL] than in the α-thalassemias [11. 2 (0. 46) g/dL] (P<0. 001) and in the β-thalassemias [11. 1 (0. 56) g/dL] (P<0. 001). The prevalence of Hb <10. 0 g/dL was statistically different among the three groups (P<0. 001). Bilirubin value was higher in the β-thalassemias [15. 2 (7. 33) μmole/L] than in the IDA students [10. 1 (3. 69) μmole/L] (P = 0. 005), while it was at statistical margin when β-thalassemias were compared with α-thalassemias [10. 7 (2. 97) μmole/L] (P = 0. 056). Among the subjects with α-thalassemia, β-thalassemia and IDA, the prevalence of hyperbilirubinemia (bilirubin value >17. 1 μmole/L) was significantly different (P = 0. 047), while variation status in the UGT1A1 gene was not statistically different (P = 0. 990). **Conclusions.** Concentrations of both hemoglobin and bilirubin are different among female students with α-thalassemia, β-thalassemia and IDA. Those differences are attributable to less Hb production and then less bilirubin formation in the subjects with IDA, not attributable to different variation status in the UGT1A1 gene among those three groups.

1836

THE XMNI POLYMORPHISM MAY DETERMINE THE MAJOR OR INTERMEDIA TYPE OF ?ETA-THALASSEMIA

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Background. The major (TM) or intermedia (TI) type of β-thalassemia is determined by the β-globin mutations (primary modifiers) as well as genetic factors which affect the α- to β-globin chain imbalance (secondary modifiers). Probable examples of secondary modifiers include the fetal hemoglobin (HbF) quantitative trait loci (QTLs): the XmnI polymorphism, Bcl11A, and the HBS1L-MYB intergenic region. **Aims.** The aim of this study was to investigate the possible correlation of these QTLs with β-thalassemia type. **Methods.** A retrospective association study was performed. To this end, >400 Iranian β-thalassemia cases were examined. To circumvent the effect of primary modifiers, only TM and TI patients having the same β-globin mutations were considered. Furthermore, β-thalassemia patients carrying α-globin mutations or deletions were excluded. Using this approach, a cohort (n=63) consisting of 27 TM and 36 TI patients with seven distinct β-globin mutations was obtained. **Summary.** Contrary to Bcl11A and HBS1L-MYB SNPs, the XmnI T/T genotype correlated strongly with β-thalassemia type in this cohort (P<0. 001).

1837

IS IRON DEFICIENCY ANEMIA ASSOCIATED WITH MIGRAINE? IS THERE A ROLE FOR ANXIETY AND DEPRESSION?

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Background. Previous studies showed that anxiety and/or depression, social and attention deficit problems were more frequent in children with iron deficiency anemia (IDA). Similarly, an association between serum ferritin levels and depression in the absence of IDA has been reported in adults. **Aims.** In this study, we determined the frequency of migraine headache in IDA patients and whether it was related to anxiety, depression and somatization. **Methods.** We

included 127 consecutive IDA patients into the study. All patients were asked validated questions about headache and migraine face to face. They were administered validated questionnaires for anxiety-depession (HADS) and somatization. The quality of life (QoL) disturbance associated with headache was marked on a 0-10 VAS. All patients gave written informed consent to participate into the study. **Results.** Of all IDA patients, 79. 5% defined headache at any time of their life. In addition, 36. 2% of all IDA patients fulfilled the criteria for migraine. IDA patients with migraine were more frequently smokers, had significantly lower hemoglobin and mean corpuscular volume (MCV) values (p values <0. 05). The IDA group with migraine had significantly higher mean anxiety score (p=0. 046), and headache-related QoL disturbance score (p=0. 021) than the IDA group without migraine. Migraine patients with aura had lower hemoglobin values (p=0. 02), higher depression scores (p=0. 005) and higher migraine-related QoL disturbance scores than others (Table 1). **Summary and Conclusions.** IDA patients have a high frequency of migraine headache. The presence of anxiety and depression have great influence on the presence of migraine in IDA patients.

Table 1. The comparison of anxiety and depression scores in IDA patients with and without migraine.

	IDA with migraine	IDA without migraine	p
n	46	81	-
Headache-related QoL disturbance (0-4)	1.89±1	1.49±0.8	**0.021**
HADS-Anxiety score	9.42±5.2	7.7±4.2	**0.046**
Anxiety (HADS-A >11)	16 (35.6)	13 (17.3)	**0.024**
HADS-Depression score	8.13±5.1	6.64±4.1	0.081
Depression (HADS-D >7)	20 (44.4)	32 (42.7)	0.85

1838

THERAPEUTIC ISOVOLEMIC ERYTHROCYTOAPHERESIS FOR IRON OVERLOAD IN HEREDITARY HEMOCHROMATOSIS, AN EFFICACY AND SAFETY ASSESSMENT

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Background. Erythrocytoapheresis (EA) removes 2-3 times more the amount of red blood cells and iron than a conventional phlebotomy. **Aims.** The aim of the study is to assess the efficacy and tolerability of isovolemic EA in patients diagnosed with hereditary hemochromatosis (HH) and iron overload. **Patients and Methods.** Observational, descriptive study. Period of study: 2001-2011. Data obtained from electronic database and clinical registries of 39 patients diagnosed with HH. Collected data: gender, age, HFE genetic mutation, total number of procedures, number of procedures till therapeutic goal (serum ferritin level < 50 ng/mL for at least 30 days), time to reach therapeutic goal, procedure related complications, use of other treatments, blood analytic parameters at the beginning and when reaching therapeutic goal. Statistical analysis was performed with informatic program. **Results.** 39 cases. M/F ratio: 28/11. Mean age 54 ± 14,3 years. EA were performed each 3-4 weeks untill response. Iron depletion was achieved in 29(74,35%) patients after a median of 15(1-108) months of treatment and a median of 11(2-42) EA sessions. Mean of total sessions per patient was 15(4-65). Previous phlebotomies in 22 (56,4%) patients. Further maintenance sessions in 27(69,2%) patients. Response were Patients did not achieved response 10 (25,64%). The mean volume of red blood cells and iron removed in each EA was 315,38±58 mL and 258mg respectively. Effects of EA on AST, Serum iron (SI), Ferritin (Ft), Transferrin saturation Index (TSI) and Hemoglobin (Hb) are shown on the table. Concomitant treatments: recombinant human erythropoietin 3, folic acid 19, iron chelators 4, vitamin B_{12} in 7. Adverse effects were well tolerated in all patients, the more frecuent: hypotension 8, catheter-related problems 7, paresthesias 4, presyncope 1 and syncope 1. **Conclusions.** In our experience as previously published, EA has proven to be an efficient, safe and well tolerated therapeutic approach for iron overload in HH. EA permits collection of massive amounts of red blood cells with mild or none complications wich is an important advantage in patients with comorbidities and leads to a better therapeutic adherence. Cost-effectiveness must be evaluated.

Table 1.

	Patients with response	Patients without response
Number of patients	29	10
HFE gen mutations	Homozygous C282Y: 17 (58.6%) Double heterozygous: 6 (20.6%) Homozygous H63D: 3 (10.3%) N/C282Y: 2 (6.9%) N/H63D: 1 (3.4%)	Homozygous C282Y: 3 (30%) N/C282Y: 3 (30%) Doble heterozygous: 2 (20%) Homozygous H63D: 1 (10%) N/H63D: 1 (10%)
AST before EA (U/L)*	43.44 ± 41.04	29.80 ± 8.13
AST after EA (U/L)*	26.72 ± 16.68	32.30 ± 23.14
Serum iron before EA (µg/dL)*	185.06 ± 56.42	207.40 ± 75.81
Serum iron after EA (µg/dL)*	96.68 ± 41.65	152.80 ± 60.16
Ft before EA (ng/mL)*	853.93 ± 913.17	747.20 ± 372.48
Ft after EA (ng/mL)*	59.13 ± 82.16	327.74 ± 194.24
TSI before EA (%)*	66.72 ± 22.29	66.41 ± 26.34
TSI after EA (%)*	29.03 ± 11.91	50.32 ± 22.72

** values expressed as mean ± SD*

1839

THE EFFICACY OF VITAMIN K2 ON BONE MINERAL DENSITY IN CHILDREN WITH BETA THALASSEMIA MAJOR

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Background. Osteopathy in thalassemia major has emerged as a topic of interest since optimized transfusion regimens and iron chealtions has dramatically improved the survival of the patients suffering from thalassemia major and increased the life expectancy. **Aims.** The aim of this prospective, monocentric pilot study was to investigate the effect of a dietary supplement with vitamin K2 (50 mcg menaquinone-7) on the bone mass in children with thalassemia major. **Methods.** After baseline investigation of the bone mass and bone formation related biochemistry (including carboxylated and undercarboxylated osteocalcin), twenty children (12 girl, 8 boys; age varied 3 to 18 years) with beta thalassemia major, who were under regular blood transfusion, chelations therapy, and followed at the Erciyes University, Medicine Faculty, Division of Pediatric Hematology, has enrolled to this study, and reinvestigated for the same parameter at the 6th and 12th months of the treatment. The results of three points were evaluated to assess the effect of dietery supplement with vitamin K2. **Results.** We detected significantly improvement of the bone mineral density and Z score at the lumbar spine and femur neck areas of the patients at the 6th and 12th months. We also found a decrease at the ratio of undercarboxylated osteocalcin to carboxyl osteocalcin, however this was not found to be at the level of significance. **Summary and Conclusions.** Although the natural course of the osteopathy is worsening or at least stabilization, we clearly find a clear positive effect of MK-7 on the bone health of the patients with TM. Supplementation of MK-7 is a physiological way, on the other hand the cost and the benefit of this vitamin seems advantageous compared with the cost and side effect of more complicated drugs such as calcitonin, clodronate, alendronate, pamidromate, and zoledronic acid. Further prospective, double-blind, multicentre, placebo controlled studies are required to elucidate the clouds of debates on the effect and cost-effectiveness of vitamin K2 on the bone disease in patients with thalassemia major.

1840

THE STUDY OF MORBIDITIES AND MORTALITY OF TRANSFUSION DEPENDENT BETA THALASSEMIA PATIENTS (SINGLE CENTER EXPERIENCE)

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Background. the improvement of Quality and duration of life of transfusion-dependent B Thalassemia patients over the last ten years discloses several complications due to the underling disorder, iron overload and the treatment with iron chelators. **Aims.** Our aim was to assess the morbidity patterns of transfusion-dependent thalassemia patients, and compare the outcomes in

relation to age of onset, type, duration and compliance to iron chelation therapy and frequency of blood transfusion. **Methods.** This retrospective study included 447 transfusion dependent β-thalassemia patients who are attended the Pediatric Hematology/oncology Clinic, Ain Shams University Children's Hospital over the last 10 years in the period between January 2000 and January 2010. Data was collected from the patients or their caregivers, as well as by reviewing follow up sheets for examinations and investigations done to detect morbidities as well as iron chelation therapies given including type, duration, compliance. Determination of mortality rate and the causes of death was also done. **Results.** Results revealed that the most common morbidities were endocrinal (44. 7%) followed by cardiovascular (41. 3%) and hepatic(40. 5%) then renal (4%). The different Iron Chelation Therapy groups showed a comparable prevalence of different morbidities. The mortality rate was 1. 5% and infection was the most common cause of death. The 5,10,20 years survival rate among the studied patients was 80%, 50%, 20% respectively. **Conclusions.** In the past 10 years the survival and morbidity rate in our center have markedly improved as result of regular blood transfusion, new iron chelators, better compliance and knowledge of the patients.

1841

THE OUTCOME OF BLOOD TRANSFUSION PROGRAMMES FOR CEREBRO-VASCULAR EVENTS IN A PAEDIATRIC POPULATION WITH SICKLE CELL DISEASE

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Background. Cerebro-vascular events (CVE) are a major cause of morbidity and mortality in sickle cell patients. The use of Trans Cranial Doppler (TCDs) measurements to predict risk of stroke in this population has been well documented (STOP trial). The Stroke prevention study in sickle cell disease showed a 90% reduction in risk of stroke with the use of transfusion programmes. Chronic blood transfusion (CBT) is the accepted standard of care for primary and secondary stroke prevention. As part of routine screening, magnetic resonance imaging and angiograms of the brain are carried out in this patient population. **Aims.** To audit the sickle cell patients on chronic transfusion programme as part of primary and secondary stroke prevention in a Paediatric Tertiary referral centre, assessing patient age, sex, haemoglobinopathy, indication for transfusion, MRI/A results, TCD results and characterise changes in TCD/MRI results with treatment and associated changes in haematologic parameters. **Methods.** Retrospective chart review of 39 patients. **Exclusion criteria.** Patients on CBT for a non-neurovascular indication. **Results.** There were 39 patients in total with a M:F ratio of 0. 86:1. All patients were HbSS. The age range was 3 - 19 with a median age of 8. 18. Indications for CBT included - Abnormal MRI/A brain 36. 1%, Abnormal TCDs 33. 3%, Clinical event (CVA/TIA/Seizure) 19. 5%, Both abnormal MRI/A & TCDs 8. 3% and Clinical event plus abnormal MRI/A or TCDs 2. 8%. 10. 3% of the patients are receiving regular exchange transfusions. 61. 5% receive top up transfusions whilst 28. 2% received initial exchange transfusions and subsequently commenced a top up programme. **Outcomes.** No patients on CBT programmes had CVE or further CVE during treatment. 15% of patients with abnormal MRI/A brain pre transfusion programmes improved, 65% remained stable while only 5% worsened on subsequent scans. 6 patients developed antibodies during treatment. All patients on top-up transfusion programmes required iron chelation treatment with subcutaneous desferrioxamine or oral deferasirox. **Conclusions.** Chronic blood transfusion is successful in the management of CVE but it has significant side effects. Nevertheless it remains our treatment of choice for neurological events in paediatric sickle cell patients.

1842

CHANGES IN IRON LOAD LEVELS, MODE OF THERAPY AND SOMATOMETRIC PARAMETERS IN YOUNG PATIENTS WITH THALASSEMIA MAJOR

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Background. The availability of new oral chelators is a major advance in the treatment of patients with transfusion-dependent thalassemia. Better efficacy of these novel therapeutic modalities, mainly due to improved compliance, is expected to result in improved hemosiderosis status and decreased complication rate. **Aims.** In this retrospective study we evaluated changes over the past decade in iron load, in the use of iron chelators, and in the somatometric parameters in young patients with thalassemia major (TM). **Methods.** All the patients with TM, followed in our unit, who were <18years old on the 31st of December 2001 (Group A) and on the 31st of December 2011 (Group B) were included in this study. The mean values of the serum ferritin levels during the past year from the time points were calculated and the iron chelation treatment during the time points was recorded. The age at the time of splenectomy was recorded. The z-scores, reflecting the somatometric parameters measured within 6 months from the time points were calculated based on 2000 CDC growth charts. The statistical analysis was performed using the STATGRAPHICS Centurion XVI software. **Results.** The results are summarized in the Table 1. During the last decade, a steady decrease of new births of patients with TM has been observed, reflecting the efficacy of Thalassemia prevention program. Splenectomy seems to play a less siginifcant role in current therapeutic approach. The use of chelators has changed from deferoxamine (DFO) to oral chelation therapy with deferiprone (DFP) and deferasirox (DFX). No statistically significant differences were observed between the two groups regarding serum ferritin levels and the patient height, weight and BMI z-scores (p=0,1, p= 0,41, p= 0,16, p=0,37 respectively). **Conclusions.** Oral chelation has become the preferable mode of treatment of hemosiderosis in young patients with TM. Despite the expected improved compliance no significant differences were observed regarding ferritin levels or the somatometric values of our patients.

Table 1.

	Group A	Group B
Patient n (male)	89 (39)	41 (22)
Age (years) (mean±SD)	12,3 ± 5,2	10,1 ± 4,1
Serum Ferritin (μg/L) (mean±SD)	2005 ± 1434	1800 ± 1548
Height z-score(mean±SD)	-0,88 ± 1,16	-0,92 ± 0,99
Weight z-score(mean±SD)	-0,24 ± 1,05	-0,12 ± 1,16
Mean BMI z-score(mean±SD)	0,32 ± 1,11	0,51 ± 0,84
Splenectomy (n)	14	1
Age splenectomy (years) (mean±SD)	9,5 ± 4,2	7,5
Treatment	Group A	Group B
DFX(%)	0	73,1
DFO (%)	91,7	3,8
DFP (%)	6,3	15,4
DFP & DFO (%)	2,1	3,8
DFX & DFO (%)	0	3,8

1843

CO-EXISTENCE OF HEREDITARY LACK OF 5-P NUCLEOTIDASE AND HETEROZYGOUS a -THALASSEMIA

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5-Pyrimidine Nucleotidase is an endo-erythrocytic iso-enzyme. Its function is to catalyze the clearance of pyrimidine ribonucleotides which comes from DNA deconstruction. The reduction of the enzyme reactivity leads to summation of pyrimidine products in the erythrocyte so that its life span increases, consistently of hemolytic anemia. 5-P Nucleotidase (5PN) insufficiency is inherited in an autosomic recessive pattern and is one of the most important causes of inherited non- spherocytic anemia along with G-6PD and pyruvate kinase insufficiencies. It is characterized by hemolytic anemia, splenomegaly and intense basophilic stippling. There are several reports in the literature concerning the reduction of reactivity of 5PN in patients with intermediate and heterozygous β thalassemia. However the co-existence of 5PN in a patient with α thalassemia is rare. We report the clinical and hematological findings of a 25 years old woman who referred to our hemoglobin prevention unit due to her pregnancy (6th week). From her past medical record, it was already known of being carrier of 5PN insufficiency . Hematological data of the propositus were as follows: Hb:10,7 gr/dL, Ht:34%, RBC:4,35*106, MCV:77 FL, MCH:24,7 pg, RDW:16,2% with ferritin levels between normal values. Microscopic examination of a stained

peripheral blood smear showed severe anisocytosis, microcytosis and basophilic stippling. The biochemical analysis of hemoglobin with HPLC showed normal values of hemoglobin A2 (HbA2:2,4%) and hemoglobin F (HbF:0,5%) while no pathological fraction of hemoglobin was observed. Due to an unclear hematologic picture of the husband, who was suspected of being heterozygous for thalassemia, we performed a genetic analysis of DNA of the patient. Molecular analysis showed that she was heterozygous of the mild mutation of α thalassemia (-α 3,7). In conclusion, the mismatches between the hematological parameters of the pregnant patient and the usual hematological phenotype which is related with α thalassemia (-α 3,7) are due to the lack of 5PN. The co-existence of hereditary lack of 5PN and heterozygous α- thalassemia is extremely interesting due to the fact that it isn't until now mentioned in the literature.

1844

LINEAR GROWTH AND CIRCULATING INSULIN-LIKE GROWTH FACTOR-I CONCENTRATIONS IN CHILDREN WITH IRON DEFICIENCY ANEMIA AFTER TREATMENT

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Objective. To assess linear growth of patients with Fe deficiency anemia (IDA) before and after in relation to their hematologic parameters and IGF-I concentration before and after treatment with iron. **Methods.** Forty children (aged 17. 2 +/- 12. 4) months with iron deficiency anemia were studied with 40 healthy normal age-matched children (controls). Patients were treated with iron syrup or drops to supply 6 mg/kg/day. Growth (weight, length and head-circumference) and hematological parameters were measured and IGF-I concentrations measured before and 3 and 6 months after treatment. **Results.** Growth parameters (weight, length and head-circumference) and hematological parameters were studied for 6 months after iron therapy. At presentation, patients with IDA had low Hb (8. 2 +/- 1. 2 g/dl), hematocrit (29 +/- 2. 8), MCV (61. 5 +/- 8. 1), and MCH (19 +/- 3. 2) which improved significantly after treatment to (11. 2 +/- 1 g/dl, 70. 6 +/- 6. 8, 23. 4 +/- 2. 9 and 18. 9 +/- 5 respectively). Before treatment children with iron deficiency they had length standard deviation score (LSDS) = - 1. 2 +/- 1, annualized growth velocity (GV) = 7. 5 +/- 2. 2, GV SDS = -1. 42 +/- 0. 6 and BMI = 13. 5 +/- 1. 2. After 6 months their LSDS = -0. 6 +/- -0. 9, annualized GV = 13. 2 +/- 4. 4 cm/year, GVSDS = 1. 7 +/- 0. 5, and BMI = 14. 2 +/- 1. 1). Circulating IGF-I increased significantly after treatment (52 +/- 18. 8 ng/ml) vs before treatment (26. 5 +/- 4. 2 ng/ml).

1845

IRON DEFICIENCY ANEMIA, CONSTIPATION AND ROLE OF MILK IN CHILDREN AGED 6 - 30 MONTHS.

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Background. Iron deficiency is the most common nutritional deficiency in the world and young children and pregnant women are at a higher risk of developing it. Anemia is defined as a Hemoglobin (Hgb)level of less than the 5th percentile for age. Constipation is defined as a delay or difficulty in defecation, present for 2 or more weeks. The most common food allergy with an incidence of 2 - 3 percent in the first year of life is cow's milk allergy. Studies have shown a relation between constipation and milk allergy in infants and young children. Children with milk allergy develop iron deficiency secondary to colitis and blood loss in stool. We believe that constipation, iron deficiency, and milk allergy may be more often interrelated than has been previously suspected and therefore that constipation and Hgb level may be related. **Aims.**To determine whether there is a relationship between constipation and Hgb in healthy infants and toddlers and the response to iron therapy. **Methods.** In this ongoing prospective study, we administered the National Health Sciences Constipation (NHS) for children to parents of 6 - 30 month old children attending the outpatient clinic who were having routine CBCs or Hemoglobin and Hematocrit done on that visit. Constipation was diagnosed by a score of ≥ 2 on the NHS questionnaire. Additional questions were asked about family history (diseases, allergies, constipation and anemia); about the child (medications, illnesses and diet); and whether the mother was breast-feeding and her diet history focusing on dairy. Comparisons were made between children with and without constipation. **Results.** Currently, there are 84 children (49 females) between 9 and 30 months old, mean 17 months. 30/84 children were constipated (score ≥ 2) and 29 children had a score of 1 on NHS survey. There was no significant difference in Hgb between children with and without constipation(p=0. 66). However, the level of constipation was inversely proportional to the Hgb level (Figure 1). There were 20 children with Hgb <11 of which 7 were constipated, and 13 were not constipated. There was a trend to a higher Hgb in children with score 0 compared to score 1 (p=0. 07). Constipation in the child (but not Hgb) was related to both of a history of allergies in the child or family and a family history of constipation. No racial differences were noted in either children who were anemic or children who had constipation. **Conclusions.** There is a weak negative correlation between the level of constipation and Hgb. A combined history of allergies in the child or in the family and a family history of constipation predict constipation in the child. Relatively few children were anemic due to iron fortification of foods. We will monitor response to iron therapy in children with anemia because we believe that constipation will negatively impact compliance. The study is ongoing and we plan to make it a multi institutional study to observe the findings in a varied population. More children with milk allergy can thus be included too.

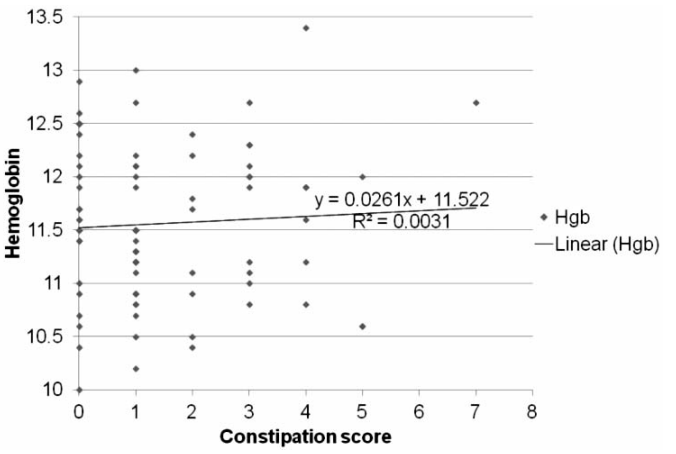

Figure 1. Constipation score and Hemoglobin (Hgb).

1846

WHAT ABOUT MILK ?

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Background. Iron deficiency anemia (IDA) strikes more than 2 billion people around the world. One of the main risk groups, with a prevalence of 20. 1%, is composed by children aged 4 months to 4 years. These children are prone to risk factors like prematurity, recurrent infections and parasitosis, but, with no doubt, the early weaning of breastfeeding, the early introduction of cow milk (CM) and it excessive consumption (>500 ml/day), are the main determinants for iron deficiency. During the first 6 months of life, breast milk meets the full-term newborn iron needs; after that period it is necessary to offer complementary food rich in bioavailable iron. CM has iron with low bioavailability and low density and an excess of calcium and proteins that interfere with the absorption of iron from other food. Thereby, consumption of CM in excess reduces the total amount of iron absorbed from the diet and replaces other possible sources of this nutrient. **Aims.** Evaluate the contribution of excessive consumption of cow milk in the etiology of children iron deficiency anemia (IDA)and draw attention to the problem of excessive consumption of milk as the main cause of IDA in the first years of life. **Methods.** Observational, retrospective and descriptive study in children with microcytic/hypochromic anemia (mhA) referred to the consultation of a Central Hematology Children's Department between January 1 and December 31, 2010. Anemia, hypochromia, microcytosis and low ferritin were defined according to the WHO guidelines (2001). Multiple variables were surveyed and data was processed using the IPSS ® v. 17. **Results.** Among a total of 148 first consultations, 64 were related to mhA and 42 out of 64 were IDA (66%). In the IDA group (62% male), the median age was of 28 months and 90% started food diversification at 4 months, 31/42 had excessive consumption of CM (mean 1000ml/day), 3/42 were ex-preterm infants, 5/42 had chronic diarrhea (3 with giardiasis), 6 (14%) had recurrent infections. Median hematological parameters (HP) at diagnosis: hemoglobin 9. 6 g/dL, MCV 66 fL, MCH 21pg, RDW 16% and ferritin 5µg/L. All were treated with oral iron (3mg/kg/day), only 7 needed iv iron. After correction of dietary errors and an average of 4-6months under oral iron, all children had normal HP and ferritin> 30. **Conclusions.** The prevalence of IDA in all our pediatric hematology first consultation was 28%. This is a high number of children if we take into account that we are a Central hospital, indicating that pediatricians and general practi-

tioners are not fully aware of this so common problem. The main cause of IDA was the excessive consumption of CM representing 66% of the first consultations for mhA. The use of CM instead of other dietary products rich in bioavailable iron, is a high risk factor for IDA and can lead to unnecessary investigations to elucidate the etiology of anemia. Questions as simple as: -what about milk? How much does he/she drinks?- can make the diagnosis, reassure parents and avoid future complications of chronic iron deficiency anemias.

1847

DEFERASIROX AND DEFEROXAMINE COMBINATION THERAPY: A CASE OF MANAGEMENT OF IRON OVERLOAD IN MYELODYSPLASTIC SYNDROME IN AN HEPATOPATHIC PATIENT.

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Myelodysplastic syndromes (MDS) are characterized by ineffective hematopoiesis, cytopenias, and a risk of transformation to acute myeloid leukemia (AML). Because the median age of the MDS onset is in the seventh decade, most patients are ineligible for potentially curative hematopoietic stem cell transplantation. Although other treatments are now available, the standard treatment for many MDS patients remains supportive care. Most MDS patients eventually become red blood cell (RBC) transfusion dependent, risking iron overload, which may lead to cardiac, hepatic, and endocrine dysfunction. Adverse effect of RBC transfusion dependence on survival was sufficiently significant that it was incorporated into the World Health Organization Prognostic Scoring System (WPSS) for MDS. Guidelines in MDS recommend chelation with an evidence of iron overload: elevated serum ferritin, iron related organ dysfunction, or chronic RBC transfusions. Deferasirox is a well tolerated oral iron chelator drug that produces relevant benefits but, because of its potential hepatotoxicity, it is not recommended for patient with hepatic diseases. A 62-year-old man, affected by HCV positive cirrhosis and MDS (Refractory Anaemia, IPSS 0. 5) started recombinant Erythropoietin therapy but it was ineffective and he underwent to a RBC transfusion program (2 blood package pro month). At a ferritin serum concentration near 700 ng/mL iron chelation therapy with deferoxamine was purposed in consideration of patient hepatic disease: compliance to subcutaneous injection was very bad, transfusion need increased exponentially until to 2 blood package pro week and serum ferritin concentration reached, in 12 months, the level of 6198 ng/mL.

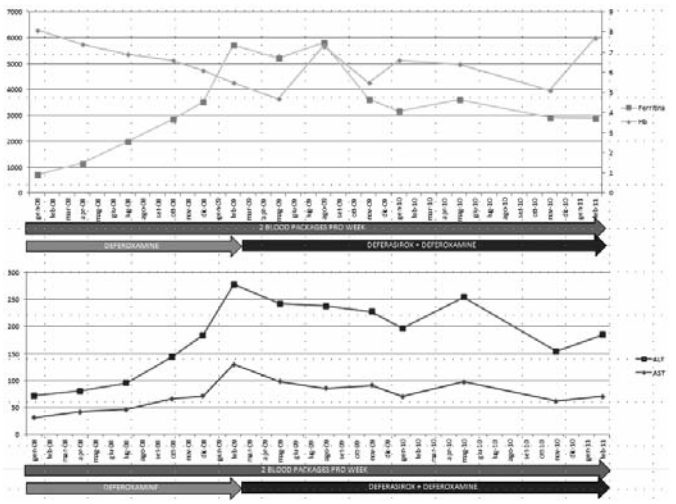

Figure 1.

Since high levels of ferritin correlate with a very dangerous condition for hepatic cells, therapy with deferasirox was started but at reduced dosage (10 mg/kg/die). Before treatment start an accurate study of hepatic, renal and cardiac functions was performed. After three months serum ferritin concentration was not modified as well as other biochemical parameters, then deferasirox dosage was gradually increased reaching 30 mg/kg/die after two months and no liver damage was observed. After five months of iron chelation therapy with deferasirox at full dosage serum ferritin concentration remained very high (5098 ng/mL). Then, considering all risks related to transfusion dependent secondary hemochromatosis, with patient informed consent, a combined iron chelation therapy with deferasirox (30 mg/kg/die) and deferoxamine (2 g/day for 5 days/week) was established and after 3 months serum ferritin concentration lowered to 3000 ng/mL. In that period, Haemoglobin concentration decreased significantly, so the patient started to receive 2 RBC package pro week and, after two years of combined iron chelation therapy, serum ferritin concentration is at a stable level nearby under 3000 ng/mL. During treatment, an accurate monitoring of hepatic, renal and cardiac functions was performed: no alterations have been noted (Figure 1). Moreover, no serious adverse event has been observed during iron chelation therapy. The patient, after a 4-years period of transfusion therapy, died from septic shock. In conclusion, management of iron overload using combined therapy with deferasirox and deferoxamine is a safe and useful therapeutic choice in critical transfusion-dependent iron overload in MDS patients with preexisting hepatic disease.

1848

GROWTH AND ENDOCRINOLOGIC COMPLICATIONS IN BETA-THALASSEMIA MAJOR IN QATAR

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Background. The combination of transfusion and chelation therapy has dramatically extended the life expectancy of thalassemic patients. The main objective of this study is to determine the prevalence of Growth and endocrine complications in children and adults with thalassemia major. **Methods.** One hundred and sixty patients entered the study. Physicians collected demographic and clinical and anthropometric data including history of therapies and pubertal status. Serum levels of 25(OH) D, calcium, phosphate, iPTH were measured. Thyroid function was assessed by free T4 and TSH. Growth hormone (GH) - insulin -like growth factor I (IGF-I) axis was evaluated in patients with short stature (< 10th percentile for age and sex). Basal gonadotrophin levels (LH and FSH) and sex steroid levels were evaluated in adolescents and adult patients. **Results.** Short stature was seen in 62% and low IGF-I in 85% of our patients. Hypogonadism was seen in 61 % of boys and girls > 14 years of age and primary hypothyroidism was present in 5%. About 24 % of patients had more than one endocrine complication with mean serum ferritin of 2818 ± 1212 micrograms/lit. In 52% of patients serum levels of 25(OH) D below 10 nmol/l and glycemic abnormalities were detected in 28% of them. **Conclusions.** High prevalence of Growth and endocrine complications among our thalassemics signifies the importance of early diagnosis and management.

Table 1. Summary of results for thalassemic patients >6 years of age.

Number	160
Age (years)	17.1+/- 10.5 years
Sex	74 F + 86 M
Short stature HtSDS < -2	62%
Low IGF-I (IGF-I < -2 SD)	85%
GHD (Peak GH < 7 ng/ml) (n = 42)	45%
Impaired Glucose tolerance (OGTT)	22.00%
Diabetes mellitus (FBG > 7.2 or 2h BG > 11.1)	6.00%
Hypothyroidism (low free T4 and/or high TSH)	5%
Hypocalcemia (Ca < 2 mmol/L)	7.50%
Hyperphosphatemia (> 1.9 mmol/L)	8.10%
Hypoparathyroidism	7.50%
Vitamin D Deficiency (25 OHD < 20 ng/ml)	52%
Impaired liver function (elevated ALT)	22.00%
Cardiomyopathy (clinical and/or echocardiography)	31%
Hypogonadotropic hypogonadism in patients >14 y (n = 79)	61%

1849

SIROLIMUS AS MAINTAINANCE TREATMENT IN AN INFANT WITH LIFE-THREATENING MULTI-RESISTANT PRCA/AIHA

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Background. Autoimmune Haemolytic Anaemia (AIHA) is a rare disease in children. Clinical symptoms of Acute AIHA can be very severe in infants, and a rapid treatment is mandatory. Children who do not respond or relapse need further therapeutic approaches but no controlled studies have ever been performed on their use. **Case Report.** We report a case of a nine month old boy with severe life-threatening resistant Pure Red Cell Anemia (PRCA)/Autoimmune Haemolytic Anemia (AIHA) within the frame of an undiagnosed immune-mediated disease. Diagnostic work-up also included both functional and genetic evaluation for FAS mediated apoptosis but did not recognize any

infective/immunological cause. The phenotype of the disease appeared to be changing overtime. Phases of prevalent intra-medullary haemolysis were alternated by more typical peripheral extramedullary one. Prednisone 3 mg/kg was administered as first line treatment in association with 2 doses Erithropoietin 450 mg/kg for the prevalence of intra-medullary haemolysis. Second and further line attack therapies of the seven relapses occurred during the next 12 months, unsuccessfully included i. v. Immunoglobulin, 3 course of Rituximab 375mg/m2, 4 doses of Cyclophosphamide 10 mg/m2, 3 courses of Alentuzumab 0. 2 mg/kg, followed by other immunosuppressive drugs administered as maintenance treatments such as Prednisone 2 mg/kg, Cyclosporine 5 mg/kg, Methotrexate 10mg/m2, Michophenolate 650 mg/m2. Massive haemolysis episodes also needed to be controlled by erithro-exchange transfusions. After failure of all above mentioned therapies, the patient was successfully treated with splenectomy followed by 3 doses of Fludarabine 20mg/m2 and Sirolimus 2 mg/m2 as maintenance treatment. During the next 18 months of follow-up, the child maintained good clinical conditions with haemoglobin levels and haemolysis indexes within the normal range. **Conclusions.** Due to the high number of immunosuppressive approaches used in a very short period, it's not possible to define which was the successful one. Frequent relapses might have been caused by the lack of both a complete de-bulk of the trigging cells and an underlying ineffective maintenance therapy. Splenectomy and sirolimus administration might have played a concomitant role in the resolution of both mechanisms. Although in our patient there was no evidence of any FAS related defect, the Sirolimus mediated lymphocytes apoptosis and the increase of regulatory T cells could have had a role in the control of an underlying undiagnosed immune-mediated disorder.

1850

HEMOLYTIC CRISIS IN AN OLD WOMAN WITH LATE ONSET FAVISM: ROLE OF AGE RELATED X- CHROMOSOME INACTIVATION AND DECOMPENSATED DIABETES

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Introduction. We describe a case of an old woman with severe hemolytic anemia in course of decompensated diabetes associated to low levels of glucose 6 phosphate dehydrogenase (G6PDH). **Case Report.** An 80 years old woman, affected by diabetes and ischemic cardiopathy, comes to our attention for weakness, jaundice, hypercromic urine, dyspnea. Blood tests show severe hemolytic anemia: Hb 6. 8 g/dl, total bilirubin 13 mg/dl, LDH 1800 UI/l, reticulocytosis, severe hemoglobinuria. Coombs tests are negative; paroxysmal nocturnal hemoglobinuria is excluded. Other exams show hyperglycemia (400 mg/dl) and ketoacidosis. Patient refers to have eaten fava beans the day before. We decide to perform red cells transfusion. After 5 days, thanks to clinical improvement, we decide to discharge the patient. After one month, she performs blood sample for dosing levels of G6PDH that result very low. **Discussion.** G6PDH deficiency is an X-linked recessive hereditary disease characterized by low levels of G6PD, a metabolic enzyme involved in pentose phosphate pathway and important for red blood cells metabolism. Patients with this disease may show non-immune hemolytic anemia secondary to different causes, commonly infection or exposure to certain drugs or chemicals. G6PD deficiency is closely linked to favism, a disorder characterized by a hemolytic reaction to consumption of fava beans. Symptomatic patients are almost exclusively male due to X-linked transmission. Female carriers can be symptomatic for unfavorable preferential inactivation (lyonization) of the X chromosome. The consequence of skewed inactivation of X-chromosome is expression of the mutant allele. Skewing of X chromosome inactivation has been linked to elderly age. Old women, heterozygous for G6PD mutations, have a higher risk to develop a G6PDH deficiency (Au et al, J of Gerontology 2006). In a diabetic G6PD-deficient subject, hemolysis may occur as result of hypoglycemia, blood glucose normalization, ketoacidosis in the African but not Mediterranean variant, and following administration of metformin or glibenclamide (Galtrey et Panthasali, J Med Case Reports 2008). Patient of described case, may have developed a G6PDH deficiency in elderly age for effect of unfavorable lyonization. Infact, during her life, ingestion of fava beans has never induced particular problems. Furthermore, hemolytic crisis could be trigger by ketoacidosis secondary to decompensated diabetes. **Conclusions.** G6PDH deficiency should be suspected in older woman with decompensated diabetes, in case of not otherwise specified non-immune hemolytic anemia.

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THE COMPARISON OF NUCLEATED RED BLOOD CELLS WITH DIFFERENT AUTOMATED BLOOD CELL COUNTERS IN PATIENTS WITH THALASSEMIA MAJOR AND ITS CLINICAL VALUE

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Background. The presence of nucleated red blood cells (NRBC) in the peripheral blood of patients with thalassemia major (TM) has long been known but its clinical important, however is less known. Modern hematology analyzers allow for rapid and accurate NRBC counts. The levels of NRBC present a good relationship with ineffective erythropoiesis. **Aims.** We wish to measure NRBC by two different automated blood cell counters and compare the results to the manual peripheral blood smear and flow cytometry as a gold standard. We also aimed to research about any relation between values of NRBC and ferritin as a marker of follow up in patients with TM. **Methods.** Samples from children with transfusion dependent TM were analyzed by two automated blood cell counters [Sysmex XE 2100 (A) and Advia 2120i (B)] and compared with the results of peripheral blood smear and flow cytometry. **Results.** Fifty one children (30 girls and 21 boys; mean age 12. 7±7. 7 year, mean ferritin value 1894±1194 ng/ml) with TM treated with regular packed erythrocytes transfusion and iron chelation agents at Erciyes University, Children's Hospital, Kayseri, Turkey, were enrolled to this prospective study. The measured mean hemoglobin values, mean reticulocyte percent, NRBC percent, NRBC count were 9. 2±1. 3 gr/dl (A), 9. 5±1. 2 gr/dl (B); 4. 6±6. 5% (A), 4. 5±5. 6% (B); 41. 3±91. 8%(A), 35. 9±83. 8%(B); 6. 1±13. 2 x 10^3/µl (A), 6. 0±14. 3 x 10^3/µl (B), respectively. The automated blood cell counters failed to measure NRBC in 1 sample (A), 5 samples (B) and the result of NRBC count found as zero in 8 samples (A), 18 samples (B). Flow cytomery showed very low values (<1%) of NRBC count in 8 samples. Linear regression analysis showed that the value of R^2 were 0. 60 (A), 0. 51 (B); whereas the y value were 4. 23+0. 15x (A), 5. 10+0. 16x (B) when compared to flow cytometry as a gold standard, respectively. **Summary and Conclusions.** This study underlined that modern hematology analyzers allow for rapid and accurate NRBC counts. Sysmex XE 2100 looks more accurate especially at low values of NRBC. Advia 2120i showed no result for low values of NRBC; however it is more accurate at high values of NRBC. We did not found any correlation between NRBC and ferritin values in two counters.

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SURVIVIN EXPRESSION IN BLOOD OF PATIENTS WITH LUNG CANCER

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Backround. Lung cancer is one of the leading causes of death worldwide, with an increasing incidence and poor prognosis. Survivin, a member of the Inhibitors of Apoptosis Protein (IAP family), has been implicated in the pathophysiology of cancer with apoptosis and cell division and its expression could be informative for the development and relapse of disease. **Aims.** In this study *SURVIVIN* expression was estimated in peripheral blood of patients with small and non small lung cancer (SCLC and NSCLC) and compared to healthy volunteers. **Methods.** Peripheral blood samples of twenty lung cancer patients, 51 with NSCLC and 8 with SCLC, and 30 healthy volunteers, as control group, were obtained. For quantitative evaluation of *SURVIVIN* mRNA expression hybridization PCR methods were used. **Results.** Survivin's mRNA levels expression in peripheral blood is:low-detected in samples of healthy volunteers (m. v. ±sem sur/abl=0,132±0,004**)** determing the basal state of expression under physiological conditions6 times higher in lung cancer patients (m. v. ±sem sur/abl=1±0,02, p=0,004) compared to controls. 9,5 times higher expressed in NSCLC patients than controls (m. v. ±sem sur/abl=1,2±0,03,p=0,001) and 5,54 times more expressed in SCLC (m. v.

±sem sur/abl=0,693±0,069, p=5,2. 10^{-5}) compared to control group. **Conclusions.** The increased expression levels of *Survivin* in patients blood with lung Cancer could be indentified to progressive disease. Also, it is possible to attribute to circulated malignant cells. Finally, determining the levels of expression of *SURVIVIN* mRNA, could provide an informative marker of evaluating the gravity, the development and the relapse of the disease and furthermore a useful tool for selecting or differentiating of the therapy strategy.

1853

CIRCULATING ENDOTHELIAL CELL NUMBER CAN PREDICT EFFICACY OF COLLECTION OF CD34+ CELLS DURING MOBILIZATION PROCEDURE

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Background. Circulating endothelial cells (CEC) in patients with hematological malignancies are assessed as non - invasive marker of angiogenesis. The other spectrum of interest is evaluation of CEC in the context of their influence on mobilization of hematopoietic stem cells (HSC) to peripheral blood. **Aims.** The aim of our study was to assess CEC and their subsets and correlate them with factors predicting successful mobilization of HSC in patients with hematological malignancies. **Methods.** Thirty eight patients were enrolled to the study (19 F, 19 M) at median age of 56,5 years. The group consisted of patients with multiple myeloma (26), lymphoma (10) and acute myeloid leukemia (2). The patients were mobilized with chemotherapy and G-CSF. For CEC evaluation venous blood samples were collected at different time points: before chemotherapy (Cht0), 1 day after chemotherapy (Cht+1), at the day of G-CSF beginning (G0), after the 1 day of G-CSF (G+1) and at the day when CD34+ cells were at least 10/µl -day of first apheresis (A). CEC were evaluated by 4 colour flow-cytometry. Circulating endothelial progenitor cells (CEPC) were defined as CD45-/CD34+/CD31+/CD133+ cells. Apoptotic CEC (apoCEC) were defined as CD146+/Annexin V+ cells. **Results.** Median (Me) number of CEC was 10,5 /µl and it decreased at G0 day (Me 8,3/µl, p=0,00006, compared with baseline), was stable at day G+1 (Me 8,4/µl, p=0,0008) and at A day (Me 8,0, /µl, p= 0,0002) as well as at A+1 day (Me 8,5/µl, p=0,007). We divided patients according to the number of days of G-CSF treatment before 1 apheresis (days of GCSF) into "early"(Me) mobilizers. We observed significantly higher baseline CEC in "early mobilizers"(Me 12,5/µl, range 6,7-21,9/µl) than in the "late mobilizers"group (Me=9,9/µl, range 3,8-21/µl, p=0,043). In "early mobilizers"we detected higher number of CEPC at G0 day (Me 1,3/µl, range 0,6-3,5/µl) than in the "late mobilizers"group (Me 0,8/µl, range 0,3-1,9/µl, p=0,05). CEPC at G+1 day was also higher in "early mobilizers"(Me=1,5/µl, range 0,8-2,2/µl) than in "late mobilizers"(Me=0,8/µl, range 0,3-1,9/µl, p=0,01). There was a trend for adverse correlation of baseline CEC number with the number of days of G-CSF (r= -0,33, p=0,06). Number of CEPC at G0 and at G+1 days correlated adversely with number of days with G-CSF administration (r= -0,42, p=0,035 and r= -0,49, p=0,04, respectively). Patients were divided according to number of aphereses needed to obtain minimal number of cells for transplantation (2 x 10^6/kg) into two groups: "highly efficient"(1 apheresis was enough) and "low efficient"mobilizers (2 or more aphereses were needed). Median ApoCEC at G +1 day was lower in "highly efficient"(Me=3,1/µl, range 1,9-5,4/µl) than in "low efficient"mobilizers (Me=5,1/µl, range 2,4-6,8/µl, p=0,02). ApoCEC at G+1 day correlated with the number of aphereses (r=0,48, p=0,038). **Conclusions.** CEC and the subsets CEPC and apoCEC measured at the time of G-CSF beginning can predict efficacy of HSC collection. CEC and the subsets as low-cost, non-invasive parameters need further evaluation in context of mobilization efficacy

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PRETRANSPLANT DONOR AND RECIPIENT CMV SEROSTATUS AND OUTCOME OF RIC ALLOGENEIC SCT FOR MULTIPLE MYELOMA IN THE ERA OF CMV PRE-EMPTIVE THERAPY

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In the era of cytomegalovirus (CMV) pre-emptive therapy, it is unclear whether CMV serostatus of donor or recipient affects outcome of allogeneic hematopoietic stem cell transplantation (allo-SCT) among patients with Multiple Myeloma (MM). Were followed retrospectively between January 2000 and January 2011, 99 consecutive patients who underwent reduced-intensity conditioning (RIC) allo- SCT for MM in our cancer centre at Marseille. All patients had serial weekly monitoring for CMV viremia using antigenemia assay (pp65 antigen) or quantitative polymerase chain reaction (PCR) at least one by week. Patients also received prophylaxis against the herpes simplex virus with oral valacyclovir (500 mg x 2/day) followed by initiation of pre-emptive therapy with Gancyclovir IV 5mg/kg x2/day for two weeks the 5mg/kg/day for other 2 weeks in patients who become positive. Median age of patients was 53 years (27-67), including 59 males and 40 females. The median age of donor was 46 years (0-71), including 46 males and 53 females. Using either matched sibling/family (73%) or unrelated donors (27%). Graft source was peripheral blood stem cells in 88 patients (89%), bone marrow in 9patients (9%), and cord blood in 2 (2%). All patients received a RIC regimen before transplantation using the combination of Fludarabine, busulfan and Anti thymoglobulin (ATG)-based (68%), or Fludarabine and Total body irradiation (TBI) based (31%). Based upon CMV serostatus, patients were classified into low risk (donor [D]-/recipient [R]-) 17 patients (17,1%), intermediate risk (D+/R-) 14 patients (14,1%), or high risk (D-/R+, 31 patients (31,3%) or D+/R+, 37 patients (37,3%)). Pre-transplant CMV seroprevalence was 68% in recipients, 51% in donors. CMV reactivation was seen in 39 patients (39%). The median time between allo-SCT and CMV reactivation was 61 days (26-318). All patients were treated with antiviral therapy with responses seen in 36 patients (92%). Twenty three of them (59%) were on corticotherapy for graft versus host disease (GvHD) treatments. Three patients (3%) developed CMV disease at a median of 38days (26-49) post allo-SCT. Only 2 patients (2%) died because CMV reactivation associated with acute GvHD. At a median follow up of 43 months (range 6-93), 43 patients (43%) are alive with a significantly higher survival among patients without CMV reactivation (27%) as compared with patients with CMV reactivation (16%; P=0. 059). CMV serostatus was the most important risk factor with incidence of 50% in the high-risk group compared with 29% in the intermediate risk and 0% in the low-risk group (P=0. 001). Figure 1. On univariate analysis, CMV status adjusted for donor type, diagnosis, disease stage, recipient age and donor age, female-to-male graft, graft source, and year> < 2006, use of ATG during conditioning and corticotherapy has no impact on Overall Survival, Progression Free Survival or Transplant Related Mortality. Other significant risk factors identified included acute GvHD (P=0. 006). On a multivariate analysis, CMV serostatus, acute GvHD remained independent predictors of CMV reactivation. In conclusion, donor and recipient CMV serologic status is a significant pre-transplant determinant for outcome in high-risk MM patients undergoing RIC allo-SCT only if associated with acute GVHD.

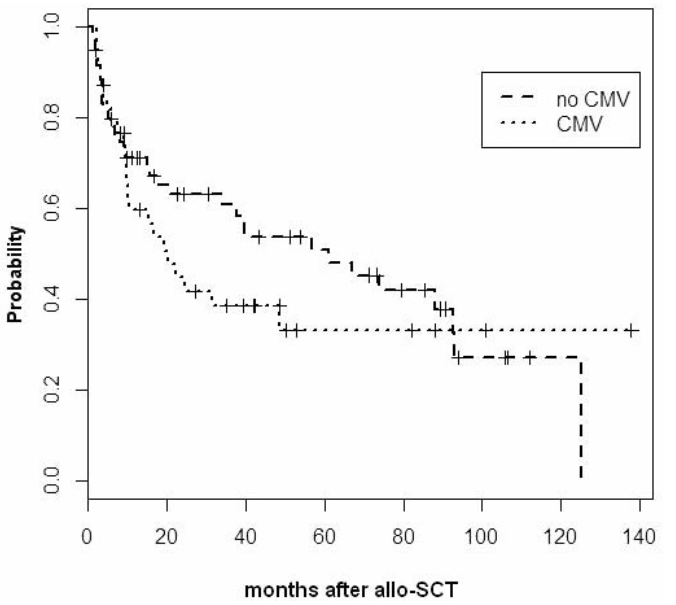

Figure 1. Overall survival OS.

1855

SEMI-AMBULATORY AUTOLOGOUS PERIPHERAL BLOOD STEM-CELL TRANSPLANTATION IN 79 PATIENTS WITH MULTIPLE MYELOMA.

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Background. There are today considerable concerns regarding the appropriate use of health care resources. Published clinical research data (Jagannah and al.

1997; Morabito and al.,2002 and Ferrara and al.,2004) reported total outpatient management which required qualified medical and nursing staff at home. We decided to realise semi-ambulatory autologous peripheral stem cell transplantation (ASCT): conditioning and reinfusion of stem cell at the hospital, five following days at home and readmission at the hospital for the management of the aplasia. . The closest published model described by Anastasia et al (2009) reports only 5% of 123 ASCT readmission before day 5. **Aims.** The purpose of this study was to analyse our experience of early discharge 2 days after high-dose melphalan followed ASCT (Day 0) and re-admission on Day +5. **Methods.** From April 2005 to July 2011, among ninety-four patients received Melphalan 200 mg/m^2 with ASCT, fifteen patients received ASCT on an inpatient basis and seventy-nine patients were treated in outpatient model. All patients presented a multiple myeloma (IgGκ=30; IgGλ=18; free light chain=17; IgAκ=2; IgAλ=5 patients). Patient were treated in first line by bortezomib (n=61) and with anthracyclines (n=31). After first line treatment, 5/79 patients were in complete response, 63 in partial remission and others patients were in partial response after a second line treatment. When they came back home, patients had the ward phone number and a date for their hospitalisation five days later. Antibiotic prophylaxis was not used. All patients received G-CSF. At Day+5, patients were hospitalised in room without positive-pressure but with protective isolation during the neutropenic period. **Results.** Of the 79 procedures eligible for our mixed inpatient-outpatient management regimen, none required early re-admission for complications. Full engraftment was achieved in all cases. Median âge was 58 years (range 31-71). Sexe ratio was 33/46. Median neutrophils count at day 1 and day 5 were respectively 3,56 (range 11,2-1,41) and 0 (range 0-88). Median platelets count at day 1 and day 5 were 214 (range 41-473) and 94 (range 94-204). During home period, digestive toxicities occurred in 28 patients: 3 oral mucositis (1 patient with grade 4 mucositis), 10 had associated diarrhea, 7 had vomiting and 6 anorexia. Two patients presented fever at home just before coming back in the hospital without septic choc. During hospitalisation, 69 patients experienced febrile neutropenia. Median duration of fever was 2 days (range 0-10). 23 patients presented clinical infection, 17 patients had documented infection by positive blood stream. Median duration of antibiotic treatment was 5 days (range 0-22). Median time to discharge (from Day 0) was 12 days (range 8-22). There was no mortality by on Day +100. **Conclusion.** Our experience of early discharge after ASCT with re-admission on Day +5 is safe and feasible with acceptable frequency of hematological and extra-hematological toxicities. The regimen allows reduced hospital stay and hence cost savings. This method generated economy of 395 hospitalisation days.

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BUSULFAN, MELPHALAN AND ETOPOSIDE FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION ON PATIENTS WITH NON-HODGKIN'S LYMPHOMA: MULTICENTER STUDY FROM CISL IN KOREA

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Background. High dose chemotherapy followed by autologous stem cell transplantation (ASCT) has become the standard approach for relapsed or high risk non-Hodgkin's lymphoma (NHL). Several different high dose therapy (HDT) conditioning regimens have been used for non-Hodgkin's lymphoma (NHL), such as BEAM (carmustine, etoposide, cytosine arabinoside, melphalan), BEAC (carmustine, etoposide, cytosine arabinoside, cyclophosphamide), and CBV (cyclophosphamide, carmustine, etoposide). Carmustine is an active drug in the HDT of NHL but the supply of carmustine is limited in some countries including Korea. Intravenous busulfan containing regimens as conditioining regimen have been used for both allogeneic and autologous stem cell transplantation in patients with hematologic and non -hematologic malignancies. The purpose of this prospective multicenter phase II study was evaluate the efficacy and safety of iv busulfan/melphalan/etoposide regimen as a conditioining regimen for high dose chemotherapy in the patients with relapsed or high risk NHL. **Methods.** Patients with relapsed or primary refractory NHL or chemosensitive high risk NHL underwent high dose chemotherapy followed by ASCT at 12 centers in Korea. The conditioning regimen consisted of iv busulfan 3. 2mg/kg/day i. v. on days -8, -7 and -6, etoposide 400mg/m2/day i. v. on days -5 and -4 and melphalan 50mg/m2/day i. v. on days -3 and -2. **Results.** Fifty one patients were enrolled onto the study. Main subgroups were DLBCL (n=25, 49%) and T cell lymphoma (n=19, 37%). At the time of ASCT, the disease status of patients was as follows: 13 patients were high risk in remission, 16 were primarily refractory to inducton therapy, 15 patients were in chemosensitive relapse. All patients had successful stem cell engraftment with a median time to neutrophil recovery of more than 500/mm3 of 10 days (range, 2 to 30 days). Platelet recovery of more than 20,000/mm3 was seen after a median of 10 days (range, 2 to 51 days) with delayed recovery in one patient. Treatment related toxicities included nausea/vomiting in 28 patients (55%), diarrhea in 28 patients (55%) and mucositis in 33 patients (65%), which were grade I or II in the majority of cases. Grade I/II hepatic toxicities occurred in 24% (n=12) and grade III in 6% (n=3). There were no VOD and treatment related death. The median duration of hospitalization for ASCT was 30 days (range, 12 to 80 days). Forty one patients (80%) achieved a complete response 1 month after ASCT, while three patients showed progressive disease. At a median follow up of 14. 7 months, 21(41%) patients exhibited a relapse or progression, while 11 patients had died of disease and one patient had died of heart failure. The estimated 2-year overall and progression free survival for all patients was 64% and 40%, respectively. **Conclusions.** This preliminary analysis suggests that conditioning regimen of i. v. busulfan/melphalan/etoposide would be well tolerated and effective in patients with relapsed or high risk NHL. Accordingly, this regimen may be regarded as an important treatment option to substitute for BEAM regimen.

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MYOCARDIAL FUNCTION ASSESSMENT IN CHILDREN UNDERGOING AUTOLOGOUS HEMATOPIOTIC STEM CELL TRANSPLANTATION

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Background and Aims. With an increasing number of survivors after bone marrow transplantation (BMT), more interest is focused on the long-term adverse effects. Subclinical cardiac involvement appears to be common in adults after BMT, but only a few previous reports of cardiac function after BMT concern pediatric patients. **Methods.** A prospective case-control study performed between September 2009 and December 2010. Nineteen consecutive patients (14 males and 5 females) with mean age 10. 73±2. 90, and normal cardiac function (LVFS >30%) undergoing autologous hematopoietic stem cell transplantation (HSCT). The underlying disease was Hodgkin lymphoma in 10 patients, Non-Hodgkin lymphoma in 5 patients and acute myeloid leukemia in 4 patients. Tissue Doppler imaging (TDI) echocardiographic measurements were obtained according to the guidelines of the American Society of Echocardiography. Informed consent was obtained. **Results.** The lateral mitral annulus in the patients before HSCT showed significant reduced mitral systolic annular (Sm) velocity (p<0. 0001), mitral early diastolic annular (Em) velocity (p<0. 0001), mitral late diastolic annular (Am) velocity (p0. 02) and prolonged isovolumetric relaxation time (IRT) (p<0. 0001) in comparison to control, while mitral Em/Am ratio and isovolumetric contraction time (ICT) remain unchanged. Comparing the same data before and after HSCT revealed significant reduced mitral systolic annular (Sm) velocity (p<0. 0001), mitral early diastolic annular (Em) velocity (p0. 0005) and mitral Em/Am ratio (p0. 004), with higher mitral late diastolic annular (Am) velocity (p0. 02) and prolonged ICT (p0. 003) and IRT (p0. 002) in patients after HSCT. Lateral tricuspid annulus in the patients before HSCT showed significant reduced tricuspid systolic annular (Sm) velocity (p0. 0002), tricuspid early diastolic annular (Em) velocity (p<0. 0001) and tricuspid Em/Am ratio (p0. 003) while higher tricuspid late diastolic annular (Am) velocity (p0. 006) and prolonged ICT (p<0. 0001) in comparison to control, whereas the ICT remain unchanged. Comparing the same data in the patients before and after HSCT revealed significant reduced tricuspid systolic annular (Sm) velocity (p<0. 0001), tricuspid early diastolic annular (Em) velocity (p0. 031) and tricuspid Em/Am ratio (p<0. 0001) while higher tricuspid late diastolic annular (Am) velocity (p<0. 0001), prolonged ICT (p<0. 0001) and IRT (p<0. 0001) after HSCT. The mid interventricular septum in the patients before HSCT showed significant reduced systolic (Sm) velocity (p<0. 0001) and Em/Am ratio (p<0. 0001) in comparison to control while early diastolic (Em) velocity and late diastolic (Am) velocity showed no changes. Comparing the same data before and after HSCT revealed significant reduced systolic (Sm) velocity (p<0. 0001), early diastolic (Em) velocity (p<0. 0001) and higher Em/Am ratio (0. 03), whereas late diastolic (Am) velocity showed no change in patients after HSCT. LV Tei index was found to be higher in patients before HSCT in comparison to control (p<0. 0001) and remains higher in the patients after HSCT (p0. 76). RV Tei index showed the

same changes observed for theLV. **Conclusions.** Subacute cardiac toxicity is common after HSCT, even in patients with apparently normal left ventricular function. TDI could detect the subtle abnormalities in the systolic and diastolic functions before HSCT and their deterioration after the HSCT which can imply that the conditioning regimen may affect cardiac function after HSCT.

1858

PREVENTIVE EXTRACORPORAL PHOTOPHERESIS USE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCIES: A PROSPECTIVE MULTICENTER STUDY

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Background. Graft versus host disease (GVHD) remains a major complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) leading to significant negative impact on patients survival and quality of life. The recent use of extracorporal photopheresis (ECP) in the treatment of chronic GVHD has resulted in very promising results especially in patients with cGVHD refractory to immunosuppressive agents. **Aims.** We conducted this prospective multicenter study to evaluate the toxicity at day 100 and efficacy of ECP introduced early (day 21 post-transplantation) in patients with hematological malignancies receiving allo-HSCT from related and unrelated HLA identical donors. **Methods.** We included adult patients of age ≤ 65 years who were in response before allo-HSCT and received reduced intensity conditioning (RIC) followed by allo-HSCT for hematological malignancies from 10/10 HLA related or unrelated donor. At day 21 post-allo-HSCT and in the absence of corticoid-resistant acute GVHD, ECP was initialized twice weekly during two consecutive weeks and then once weekly the weeks later, in total, 8 ECP sessions had to be done. At day 100 post allo-HSCT, ECP toxicity evaluation was done according to the NCI/NIH common toxicity criteria (version 3). Other outcomes on efficacy and survival were also assessed. **Results.** Between June 2009 and March 2011, 10 patients were included, 6 males and 4 females of median age 61 years [54-65]. There were 4 AML (all in CR1 before allo-HSCT), 2 NHL (1CR1 and 1CR3), 2 CLL (1VGPR3 and 1CR3) and 2 MM (1 CR2 and 1 PR2). All patients received Fludarabine - Busilvex - ATG based RIC. Peripheral blood stem cell (PBSC) was used in all patients, 5 from HLA identical related donors and 5 from HLA identical unrelated donors. Patients received allo-HSCT after a median time of 6. 6 months [4. 5-8. 7] from diagnosis. Sex matching was: F→M:2, F→F:2, M→F: 2 and M→M:4. All patients engrafted, the median time to neutrophils and platelets recovery were 17. 5 days (13-19) and 9. 5 days (0-13) respectively. At day 21, no corticoid-resistant aGVHD were observed, ECP was administered for all patients; 8 patients completed the 8 ECP sessions while one patient received 4 sessions (due to GVHD activation) and one patient received 5 sessions (due to EBV lymphoproliferation). There were 2 patients with aGVHD (1grade I at day 17 and 1 grade IV at day 37). cGVHD was observed in 4 patients [3 limited (at days 100, 141 and 180) and 1 extensive (after DLI + lenalidomide) at day 365. After a median follow-up of 13. 3 months (2-24), one patient died due to septic choc while relapsing, 9 patients are alive, 7 are in CR, 1 in PR and 1 in relapse, none of the patients present active chronic GVHD. The ECP was well tolerated, there were only 2 serious adverse events (SAE) with grade ≥ 4 (one febrile neutropenia and one septic choc) both not related to ECP procedure, **Conclusions.** ECP has resulted in very acceptable rates of GVHD. This procedure has been well tolerated; similar larger prospective studies are needed to validate its use in a preventive way.

1859

ORAL FLUDARABINE WITH LOW DOSE TBI CONDITIONING FOR ALLOGENEIC PBSC TRANSPLANT ACHIEVES EFFECTIVE DONOR ENGRAFTMENT WITH REDUCED RISK OF SEVERE NEUTROPENIA

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Background. Allogeneic HSCT conditioned withlow dose TBI and intravenous (i.v.) fludarabine results in reduction in NRM, however, is associated withhighgraft rejection rate. Use of bone marrow instead of G-CSF mobilisedPBSC, and low CD 34+ and CD3+ cell doses are the factors associated withhigher graft failure rates. RICHSCT withoral fludarabine, in combination witheither melphalan or busulfan, results in adequate donor engraftment. However, no data exist withregard to donor engraftment kinetics in patients athigher risk of graft rejection i. e. treated withoral fludarabine in combination witha single fraction of 2 Gy TBI. **Aims.** To study early donor chimerism in allogeneic HSCT usingRIC withoral fludarabine and 2 Gy TBI. **Methods.** Patients withAMLand MM who had at least an 8/8-matching (HLA - A, - B, -Cw and -DR) sibling or an unrelated adult donor were transplanted withPBSCs following conditioning withoral fludarabine 48 mg/m^2/day days -4 to -2; and 2 Gy TBI on day 0. Post transplant immunosuppression consisted of cyclosporin 3 mg/kg b. d. starting day -3 and mycophenolate mofetil 15 mg/kg b. d. starting 4-6hours after stem cell infusion. Donor/recipient mixed chimerism was assessed based on peripheral blood lymphoid (CD3+) and myeloid (CD33+) cells, by short tandem repeat polymerase chain reaction on days 30, 60, 90 and 180. Inadequate CD3+ chimerism (< 90% CD3+) was managed with early withdrawal of immunosuppression &/or infusion of donor lymphocytes. **Results.** Seven males and 7 females underwent HSCT for either MM (N=12) or AML(N=2). For MM patients, allogeneic HSCT was part of a planned tandem autograft-allograft procedure, using a sibling (n = 7) or unrelated (n = 7) donor. Five unrelated donors were mismatched either at HLA-DP (4) or -DP and -DQ(1). Six donor recipient pairs were sex mismatched. The median CD34+ cell dose was 7. 11 x 10^6/kg (range: 2. 39 - 23. 96 x 10^6) and the median CD3+ dose was 225 x 10^6/kg (range: 136 - 469 x 10^6). No grade III/IV gastrointestinal toxicity was seen. Median neutrophil nadir count was 0. 58 x 10^9/L, which was significantly lower compared tohistorical data using i. v fludarabine at equivalent dose (Figure 1). Red cell transfusion was required in four patients and platelet transfusion in two. No mortality was seen by day +180. Six patients achieved ≥90% CD3+ donor chimerism by day 30, 8 by day 60 and 10 by day 90 (3 patients not yet reached this milestone). In CD 33+ lineage, 12 patients achieved ≥90% donor chimerism by day 30 and in all 14 by day 60. The donor chimerism kinetics is shown below (Figure 2). One patient each required withdrawal of immunosuppression and donor lymphocyte infusion to improve donor chimerism, which was effective. **Conclusions.** Oral fludarabine and 2 Gy TBI based conditioning is well tolerated withless severe neutropenia than i. v fludarabine. The lymphoid engraftment is delayed but similar to i. v. fludarabine. Thus, oral fludarabine appears to be an effective and safe conditioning for outpatient allogeneic HSCT.

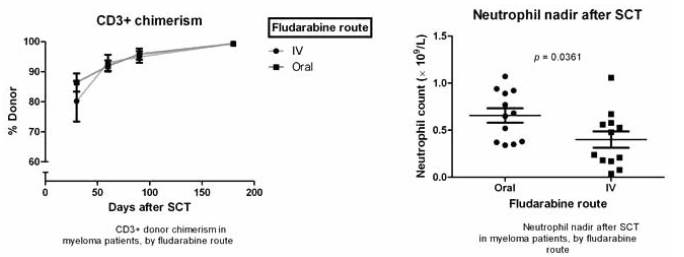

Figure 1.

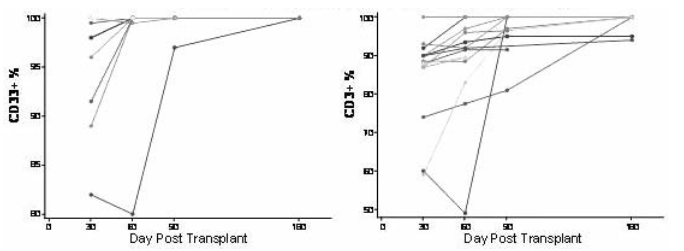

Figure 2. Chimerism kinetics and neutropenia.

1860

LONG-TERM FOLLOW-UP OF REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR REFRACTORY OR RELAPSED FOLLICULAR LYMPHOMA

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Background. Although allogeneic hematopoietic stem cell transplantation (HSCT) is considered the only curative treatment for refractory or relapsed follicular lymphoma, transplant-related mortality (TRM) greatly interferes with the success. A variety of reduced-intensity conditionings (RICs) have been used to reduce TRM, but an optimal conditioning for follicular lymphoma has not been ful-

ly established. **Aims.** To evaluate the safety and efficacy of RIC for follicular lymphoma, we retrospectively evaluated the long-term outcome of allogeneic HSCT for follicular lymphoma with a RIC regimen consisting of fludarabine and melphalan. **Patients and Methods.** Nineteen patients (age: 34-58) with relapsed or refractory follicular lymphoma were conditioned with fludarabine (125 mg/m^2) and melphalan (140 mg/m^2), and received bone marrow or peripheral blood stem cells from a human leukocyte antigen (HLA)-identical sibling (n=6) or bone marrow from an HLA-matched unrelated donor (n=13). For the prophylaxis of graft-versus-host disease (GVHD), cyclosporine A or tacrolimus with short-term methotrexate was given. **Results.** There were no early deaths before engraftment, and all patients achieved engraftment. Three patients died of extensive-type chronic GVHD (n=2) or bacterial infection (n=1) without disease progression. With a median follow-up period of 75. 2 months (range: 33. 3-111. 9 months), 16 patients were alive without disease progression. Both the 5-year overall and progression-free survival rates were 84. 2% (95% CI: 67. 7%-100%). **Conclusions.** These results strongly suggest that allogeneic HSCT with RIC using fludarabine and melphalan could be a promising treatment choice for refractory or relapsed follicular lymphoma. For further improvement of the outcome, optimal management of GVHD should be explored in this setting.

1861

DIFFERENT RISK FACTORS AND ECONOMICAL RESOURCES RELATED TO BK VIRUS-ASSOCIATED HEMORRHAGIC CYSTITIS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction. Virus-associated hemorrhagic cystitis (HC) is a major cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (allo-HSCT). **Aims.** To reveal the different risk factors associated to the occurrence of BK virus-associated HC (BKvHC) and evaluate its economical impact. **Materials and Methods.** We conducted this retrospective analysis on patients who received allo-HSCT for any hematological malignancy between January 2007 and December 2011 at our institution. The incidence of BKvHC was assessed. Different transplantation and disease characteristics were evaluated to study their impact on the BKvHC occurrence. A medico-economical study including days of hospitalization and transfusions was done. **Results.** Among 304 patients who received allo-HSCT, there were 42 (14%) who developed BKvHC. The median time of occurrence was 37 days (9-361) post allo-HSCT and it lasted for a median time of 20 days (1-155). Cidofovir treatment was initiated in 38 (90%) patients resulting in 63% of clinical response at 2 weeks and 71% at 5 weeks, among them 63% were cured. In multivariate analysis, the factors associated with a higher risk of developing BKvHC were: transplants from unrelated donors including cord blood transplants (OR 2,8; 95% CI 1. 30-6. 38; p < 0. 01) and the existence of HLA mismatch (OR 2. 3; 95% CI 1. 09-5. 11; p < 0. 05). Patients with BKvHC needed significantly longer time of hospitalization (65. 8 vs 45. 1 days; p < 0. 001), more red blood cells and platelets transfusions units [(21 vs 6. 3; p < 0. 001) and (28. 8 vs 9. 2; p < 0. 001) respectively]. **Conclusions.** We showed that donor type and cells source including the HLA matching play a major role in the occurrence of BKvHC. The consideration of these parameters in the allo-HSCT settings should have a positive outcome on its incidence and a significant medico-economical impact especially on hospitalization time and transfusions. Larger prospective studies are needed in order to validate our findings.

1862

WITHDRAWN

1863

USE OF MESENCHYMAL STEM CELLS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: Results OF COMPASSIONATE USE IN A SINGLE CENTER

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Background. In recent years the use of mesenchymal stem cells (MSC) for the treatment of complications in the alogeneic hematopoietic transplant setting is increasing. Graft-versus-host disease (GVHD) is the most frequent indication for this treatment, but a number of other complications have been also treated such as graft failure or haemorrhagic cystitis. **Aims.** The aim of this work is to analyse the outcome of patients treated in our institution with MSC in the compassionate use program. **Patients and Methods.** Patients: 23 patients have been treated with MSC in our institution in the compassionate program during 2010 and 2011. Median age was 51 years (range 2-62). Three children received an allogeneic transplantation for non-neoplastic diseases, the remaining had been diagnosed of an haematological neoplasia (6 acute leukemias, 5 lymphomas, 2 chronic lymphocytic leukemia and 7 myelodysplatic syndrome or myeloproliferative disorder). The indication for MSC use was: refractory acute GVHD-11, refractory extense chronic GVHD-2, graft failure-3, GVHD with cytopenia-2, and 5 patients had a cytopenia without other signs of GVHD. All patients received bone marrow derived MSC from a third party donor. MSC EXPANSION AND ADMINISTRATIONCells were expanded in the GMP facility of our institution according to established protocols. Briefly mononuclear cells were obtained by density gradient and platted in culture flasks with culture medium supplemented with platelet lysate. After confluence was reached cells were trypsinized and replated for 2-3 passages. Then cells were frozen in individual doses. Informed consent was obtained from both, patients and donors according to institutional rules. **Results.** A total of 112 infusions have been performed. Cells were thawed and iv administered after dexchlorpheniramine and hydrocortisone treatment. No side effects were reported during or after infusion. A median 4 infusions (range 1-12) have been administered. The median number of cells in each dose was 1x10^6/Kg of patient (range 0,6-3). Globally 19 out 23 treated patients showed some response. The results are shown in Table 1. **Conclusions.** MSC administration is safe as treatment of severe complications in the allogeneic stem cell transplantation setting. The number of cells and of doses needed to obtain the optimal response need to be determined.

Table 1.

Number of patients that achieved complete response	Number of patients that achieved complete remision	Number of patients that achieved partial response	Number of patients with no response
Acute GVHD n=11	5	4	2
Chronic GVHD n=2	0	2*	0
GVHD + cytopenia n=2	1	1	0
Graft failure n=3	2	0	1
Peripheral cytopenia n=5	4**	0	1
Total n=23	12	7	4

* One of them reached CR of gut GVHD but no response of liver GVHD

** Three out 4 patients relapses and were threated with a second course of MSC. Two of them maintained CR (3 and 4 months respectively until now)

1864

CONVENTIONAL VS. LARGE VOLUME APHERESIS FOR ALLOGENEIC AND AUTOLOGOUS STEM CELL TRANSPLANTS

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Background. The collection and clinical use of adequate number of stem cells (SCs), harvested by leukapheresis, is necessary for effective treatment of hematological disorders by transplant. **Aims.** The goals of this study were: to obtain an optimized protocol for harvesting sufficient quantity of viable SCs and to compare the hematopoietic reconstitution after autologous transplants in different clinical settings. **Methods.** In this study, SC transplants - 112 allogeneic from matched sibling healthy donors and 128 autologous - were performed in the management of patients with severe aplastic anemia, leukemias (ALL, ANLL, CML), multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma, and extragonadal non-seminal germ cell tumor. Cell mobilization was achieved with rHuG-CSF (5-10 mg/kgbm/day and 12-16 mg/kgbm/day in allogeneic and autologous setting). SC-apheresis procedures (generally one and occasionally two) were performed using blood cell separators COBE-Spectra and Spectra-OPTIA (CaridianBCT, USA). The first SC-apheresis was accomplished when the leukocyte cont was 5-10x10E9/L and CD34+ count 30-50/mL in peripheral blood

(autologous) or on the 5th day 4-5 hour after the last rHuG-CSF administration (allogeneic). The processed blood volume during one apheresis was 24. 3 L for one SC transplant in average. **Results.** Using a minimal target dose of CD34+ cell count (3x10E6/kgbm), performing one apheresis procedure for 86% recipients sufficient number of SCs were obtained. The MNC yield was 10. 1x10E8/kgbm in allogeneic and 8. 1x10E8/kgbm in autologous setting. The mean CD34+ yields for allogeneic and autologous transplants were 8. 1x10E6/kgbm and 6. 5x10E6/kgbm, respectively. The ratio of primitive CD34+ cell subpopulation CD34+/CD90+ was higher (1. 7±1. 5% vs. 1. 3±0. 8%) in autologous SC harvest. Hematopoietic reconstitution was achieved on the 10. 4th vs. 12. 4th day for leukocytes and the 14. 05th vs. 17. 4th day for platelets when allogeneic vs. autologous SC transplants were applied. **Summary and Conclusions.** Improved CD34+ cell yield with acceptable primitive CD34+ subpopulation ratio was obtained, as well as consequential rapid hematopoietic reconstitution were observed when large volume apheresis was performed using higher (12-16 mg/kgbm/day) rHuG-CSF doses.

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IRF AND IPF AS PREDICTORS OF HAEMATOPOIETIC RECOVERY AFTER ALLOGENEIC TRANSPLANTATION IN CHILDREN AND ADULT PATIENTS

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Haematopoietic progenitor cell transplantation may be the treatment of choice for patients with malignant haematological diseases. In the post transplant period it is particular relevant to evaluate the haematological engraftment of these patients. Last generation haematology analysers offer an alternative way to evaluate the PB immature fractions which may also give indications of haematopoietic recovery. Amongst these, the immature reticulocyte fraction (IRF) and immature platelet fraction (IPF), in peripheral blood samples, could play an important role as early indicators of transplant's success. Traditionally, neutrophils (NEUT) (D500 NEUT>0. 5 x 10^9/ L) and platelets (PLT) (D20 PLT>20 x10^9/L) counts are used to evaluate the haematological engraftment of transplanted patients. The aim of the present study was to evaluate the predictive value of IRF (D IRF>10%), and IPF (D IPF>7%), in the haematological recovery of adult (n=60) and paediatric (n=21) patients undergoing allogeneic transplantation, together with the classical parameters. Related or unrelated progenitor cell grafts were obtained: Cord Blood (CB) and Bone Marrow (BM) for paediatric patients and Peripheral Blood Stem Cells (PBSC) for both groups of patients, who were followed since transplant day throughout all hospitalization period. Patient Peripheral Blood (PB) samples were collected in EDTA tubes and analyzed in the automatic hematology analyzer Sysmex XE-5000. The classical parameters of the CBC, namely the concentration of leucocytes, NEUT and PLT were obtained together with the new parameters IRF and IPF. The haematopoietic recovery of both groups of patients is summarised in Table 1, comparing median days to recovery for classical parameters and new parameters.

Table 1. Haematopoietic recovery of adults and paediatric patients.

	NEUT	PLT	IPF	IRF
Adults (n=60)				
All	14(11-28)	11(8-40)	10(6-30)	10(4-33)
Relat (47)	14(11-18)	10(8-29)	9(6-16)	10(4-14)
Unrelat (13)	15(12-28)	21(11-40)	13(9-30)	15(9-33)
Paediatric (n=21)				
All	15(10-22)	15(11-32)	11(8-22)	13(9-22)
Relat (5)	15(12-16)	13(12-19)	10(8-17)	11(10-13)
Unrelat (16)	15(10-22)	17(11-32)	12(8-22)	13(9-22)
CB (4)	16(13-18)	22(13-30)	14(9-22)	11(9-17)
BM (4)	17(12-19)	18(12-27)	12(8-17)	15(11-22)
PBSC (13)	15(10-22)	14(11-32)	11(8-20)	12(10-19)

For adult patients we observed an anticipation of IRF recovery by 4 days in comparison with NEUT recovery (10 *vs* 14 days), and by one day of IPF in comparison with PLT (10 *vs* 11 days). Patients receiving unrelated grafts experienced further delay in their PLT recovery (13 *vs* 21 days). For paediatric patients we observed an anticipation of IRF recovery by 2 days in comparison with NEUT recovery (13 *vs* 15 days), and by 4 days of IPF in comparison with PLT (11 *vs* 15 days). All paediatric patients receiving any sort of unrelated grafts experienced further delay in their recovery, in particularly CB recipients. In this subgroup we observed an anticipation of IRF recovery by 5 days in comparison with NEUT recovery (11 *vs* 16 days), and by 8 days of IPF in comparison with PLT (14 *vs* 22 days). With this study we conclude that IRF and IPF predict the haematopoietic recovery of patients. For allo-transplanted patients receiving unrelated grafts that prediction is even more noteworthy and clinically very relevant. The results observed in children transplanted with CB allografts are encouraging as they may be the first indication of the success of the transplant, although a large number of patients is needed to confirm these findings. From this study IRF and IPF emerge as potential useful tools in haematopoietic transplantation.

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ASSESSMENT OF LIVER FIBROSIS WITH FIBROSCAN IN THALASSEMIA MAJOR POTENTIAL CANDIDATES FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background and Aims. To assess the diagnostic accuracy of Fibroscan in patients with β-thalassemia major (TM) who are candidates for hematopoietic stem cell transplantation. **Methods.** The study contained 58 patients prospectively recruited with TM (median age: 8 years; range: 2. 6-20 years; male: 65. 5%). All patients underwent liver stiffness measurement using Fibroscan. The results were expressed in Kilopascal (KPa) units. The specimens were scored according to the Ishak system. The diagnostic value of TE was compared with the histological fibrosis stage using linear discriminant analysis (the area under the receiver operating characteristic curves (AUROCs)). **Results.** Twenty -six patients (44. 8%) had mild fibrosis, 12 (20. 7%) had moderate and 2 (3. 4%) had severe fibrosis. Fibroscan values were significantly correlated with Pesaro's classification for thalassemia (p <0. 001), iron deposition (p<0. 037) and fibrosis stage (p=0. 008). Median Fibroscan values in patients with severe (stage 3-5), mild or no fibrosis were 4. 5 (3. 0-13. 0) kPa and 4. 0 (2. 5-9. 0) kPa (P = 0. 000), respectively. To predict high fibrosis stages (stage ≥ 3) with cut-off of 4. 35 kPa, AUROC was 0. 670 (95% confidence interval [CI]: 0. 508-0. 833) with 76. 9% sensitivity (95% CI: 70. 8-81. 8) and 57. 8% specificity (95% CI: 53. 3-60. 3). **Conclusions.** Fibroscan appears to have a high diagnostic accuracy of fibrosis stage in TM patients who are potential candidates for hematopoietic stem cell transplantation . It can also be used as a valuable tool to follow-up the liver status in patients with TM after transplantation. Further studies with more cases are required to achieve reliable results for practical application.

1867

PROGNOSTIC SIGNIFICANCE OF PRE-TRANSPLANT RESPONSE STATUS IN PATIENTS WITH MULTIPLE MYELOMA

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Background. There are several prospective and retrospective studies exploring the impact of pre-transplant response status on disease outcomes in patients with multiple myeloma. However, Conclusions of these studies are controversial. While almost a half claims that it has a prognostic impact on myeloma endpoints, others found no association. **Aims.** We aimed to investigate the impact of pre-transplant response depth on myeloma outcomes. **Methods.** We retrospectively analyzed the data of a total of 56 patients with multiple myeloma who underwent autologous stem cell transplantation (SCT) between 2000 and 2010 in Marmara University Hospital, Division of Hematology. Response status was defined according to IMWG criteria. Primary endpoint was time to progression (TTP: time elapsed between transplantation and progression or progression-related death). Secondary endpoints were progression-free survival (PFS: time from transplantation to progression or any death), time-to-next-treatment (TNT: time from transplantation to next treatment, relapse, or any death), and overall survival (OS: time from transplantation to any death or last contact). Post-hoc power analysis revealed that study show a hazard ratio (HR) of 0. 5 with a power of 72% and an overall two-sided type I error of 5%, with use of a two-sided log-rank test. **Results.** Time-to-transplantation, median follow-up, and treatment related mortality were 12 (6-36) months, 32 months, and 3. 6%, respectively. For all cohort, median PFS, TTP, TNT, and OS were 25, 32, 47, and 84 months respectively. 5- and 10-year OS were 60. 9%, and 26. 6%. The proportion of patients who achieved ≥ very good partial

response (VGPR) before transplantation was 41. 9%. Post-transplant ratio of patients with ≥VGPR estimated to be 75. 8%. There were no significant differences between patients with a pre-transplant response ≥VGPR and partial response (PR) in terms of PFS (median 33 vs 24 months, p= 0. 3), TTP (not reached vs 25 months, p= 0. 2), TNT (not reached vs 33 months, p= 0. 09), and OS (not reached vs 71 months, p= 0. 8) (Figure 1). **Conclusions.** Patients with multiple myeloma who achieve PR or ≥VGPR before transplantation have similar disease outcomes. Since only 30% of patients will have a ≥VGPR with novel agents, at least PR is a reasonable pre-transplant treatment goal.

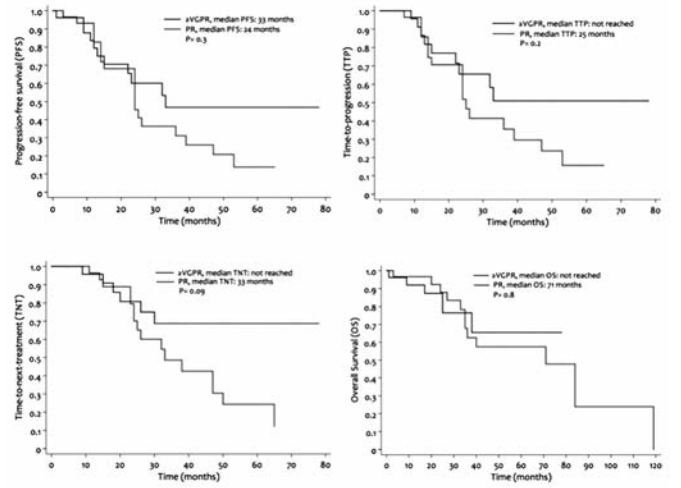

Figure 1.

1868

WHAT IS THE CONTRIBUTION OF HOST-DERIVED CMV IMMUNITY AFTER ALLOGENEIC TRANSPLANTATION FOLLOWING NONMYELOABLATIVE CONDITIONING?

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Background. It has been suggested that host-derived CMV-specific immunity could persist in patients given grafts following nonmyeloablative conditioning. **Aims.** In the current study, we challenged this hypothesis by assessing chimerism levels among CMV-specific CD8+ T cells around days 40, 100 and 180 after allo-HCT in a cohort of 24 patients given allogeneic grafts after nonmyeloablative conditioning. **Methods.** Data from 24 patients given unmanipulated peripheral blood stem cells (PBSC) after nonmyeloablative conditioning were included in this study. Detection of CMV-specific CD8+ T cells was performed on previously cryopreserved peripheral blood mononuclear cells (PBMCs) according to the combinatorial encoding method of CMV pMHC multimers. CMV specific and unspecific CD8+ T cells were sorted into distinct tubes. DNA was isolated from cell pellets to assess their chimerism. **Results.** Only 4 of 17 CMV-seropositive recipients given grafts from CMV-seronegative donors had a higher by >25% proportion of cells of recipient origin among CMV-specific CD8+ T cells (ranging from 32. 4 to 100%) than among remaining CD8+ T cells on day 100 after transplantation. The 2 patients with CMV-specific CD8+ T cells that were >99% of recipient origin on day 100 had relatively high counts of CMV-specific CD8+ T cells on that day (13. 1 and 14. 7 cells/μL). **Conclusions.** these results demonstrate that high numbers of CMV-specific CD8+ T cells of recipient origin could be present after allo-HCT, although in a minority of nonmyeloablative recipients.

1869

FEASIBILITY OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA OLDER THEN 65 YEARS

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Introduction. Autologous peripheral stem cell transplantation is the standard therapy for the treatment of lymphoma into the young patient who expresses poor prognostic factors at diagnosis or at relapse (high IPI). Only few data are available on the feasibility and outcome of such procedures in patients over 65 years. **Aims.** We conducted a single centre, retrospective, study on the feasibility and the results of autologous transplantation in patients carrying a lymphoma, aged 65 or over, compared to younger patients. **Patients and Methods.** Over a period of 10 years we identified 151 patients with lymphoma who performed autologous stem cell transplantation at BREST (France) transplantation centre. 95 patients were under 65 years and 56 patients were older. We compared two populations in terms of number of units of red blood cells and platelets transfused, number of days of neutropenia and incidence of complications related to transplantation procedure. **Results.** No statistically significant difference was seen, between the two populations, for the number of units transfused (CG: 2. 9/3. 7 p - 0. 21 and CPA: 4. 51/4. 18, p - 0. 58). The only significant difference (p - 0. 0006) is the duration of aplasia: 11. 22 days for younger patients and 11. 81 days in elderly patients. The mortality rate associated with the procedure (MRT) is similar in both groups of patients with 2 deaths in each arm. There was no statistically difference between the two populations, regarding the OS (40,91/38. 33, p-0. 11) and DFS (39. 4/33. 18, p-048). **Conclusions.** In our study we found that there was no significant difference between the two populations (over or under 65 years) for the number of units of blood and platelet transfusions. Only the difference of days of neutropenia appears to be significant and it is for the young patient. The autograft procedure remains under certain conditions, a possible treatment option for patients older than 65 years treated for lymphoma.

1870

HEMATOLOGICAL RECOVERY AND EARLY RISK INFECTION: EFFECT ON THE EVOLUTION AFTER AUTOTRANSPLANTATION

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Background. Absolute lymphocyte count (ALC) < or=500/μl on day(D)15 post-autologous haematopoietic stem cell transplantation (HSCT) has been proposed as an independent risk factor in the outcome undergoing HSCT in patients (P) with hematologic malignancies **Aims.** The aim of study is to compare the influence of ALC, absolute neutrophil count (ANC) and absolute platelet count (APC) recoveries on the risk of early infection (REI) post-HSCT (D< or =30) and survival **Methods.** Medical records of 113 P (59 non Hodgkin lymphoma (NHL), 22 Hodgkin lymphoma (LH) and 32 multiple myeloma (MM)) receiving autologous-HSCT between January 2006 and March 2012 in La Paz University Hospital were reviewed. The analysis of the relationship between hematological recovery post-HSCT (ALC>500/μl on D15, APC>20000/μl on D15 and ANC>500/μl on D12), REI, overall survival (OS) and progression-free survival (PFS) was performed by Mann-Whitney U test, Chi-Square test and log rank test, we also applied survival curves of Kaplan-Meier and Cox regression model **Results.** Median of days required to ALC>500/μl was 14 (range: 12-17), ANC>500/μl was 14 (range: 12-16) and APC>20000/μl was 12(range: 10-15). We found statistically significant differences between REI and ALC500/μl at that D (60% vs. 36%, p=0. 02; OR 2. 69 (CI95%:1. 23-5. 85)) and ANC500/μl at that D (37% vs. 61%, p=0. 0017; OR 2. 73 (CI95%: 1. 22-6. 07)). We found no significant association between REI and APC20000/μl (63%vs. 41%, p=0. 09; OR 2. 50 (CI95%: 0. 95-6. 57)). There is a significant relationship between post-HSCT survival and hematological recovery so that, ALC>500/μl on D15 (n=67) is associated with better PFS and OS, than ALC below this limit (n=46): PFS 65 months (m) (CI95%: 58-72) vs. 31 m (CI95%: 20-41), p<0. 0001 and OS126 m (CI95%:117- 134) vs. 54 m (CI95%: 55-74), p<0. 01; APC>20000/μl on D15 (n=90) is associated with better PFS but no OS, than APC below this limit (n=23): PFS 56 m (CI95%: 48-63) vs. 33 m (CI95%: 18-48, p<0. 007 and OS 118 m (CI95%:108-129) vs. 61 m (CI95%: 47-75), p<0. 116; ANC>500/μl on D12 (n=39) is not associated with better PFS or OS, than ANC below this limit (n=74): PFS 48 m (CI95%: 40-57) vs. 56 m (CI95%: 45-68), p<0. 217 and OS 119 m (CI95%: 104-134) vs. 71 m (CI95%: 65-77), p<0. 787. Multivariate analysis showed that P those to reach ALC 500/μl after D15 and P with HL or MM have higher risk of relapse with HR 4. 56 (CI95%: 2. 3-9. 04), p<0. 001 and HR 2. 56(CI95%:1. 17-5. 63), p=0. 019 **Conclusions.** 1) The study shows that P with ALC<500/μl on D15 have 3 time more probability of REI and P with ANC<500/μl on D12 have 2 time less probability. 2) We confirm positive prognosis impact of ALC>500/μl on the evolution after autotransplantation. 3) No achieve APC 20000/μl on D12, is a worse prognosis factor for PFS but no affect to OS or REI 4) NHL has lower risk of relapse after autologous- HSCT.

1871

AUTOLOGOUS PERIPHERAL BLOOD HEMATOPOIETIC STEM CELL TRANSPLANTATION(HSCT) FOR HEMATOLOGIC MALIGNANCIES IN SHIRAZ,SOUTH OF IRAN

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Background. Hematologic malignancy currently represents the main indication for HSCT. clearly, autologous and allogeneic HSCT are established therapies in many of hematologic malignancies. High dose therapy(HDT) supported by autologous HSCT are the preferred choice for lymphoproliferative disorders and multiple myeloma. **Aims.** Bone marrow transplantation was established in Shiraz University of medical sciences in1993. since 2003, stem cell sources from bone marrow changed to peripheral blood in our center and we started peripheral blood autologous stem cell transplantation for hematologic malignancies. In this article we have presented results of autologous stem cell transplantation in our center during 7 years. **Methods.** Between Jan 2004 and Nov 2011, 253 patients with diagnosis of hematologic malignancies including Hodgkin's lymphoma(HD), non-Hodgkin's lymphoma(NHL),Acute myelogenous leukemia (AML)and multiple myeloma(MM)underwent autologous peripheral blood stem cell transplantation in our center in Shiraz university of medical sciences. Patients were treated by intensive chemotherapy followed by reinfusion of non-cryopreserved autologous stem cells. The pretransplant conditioning chemotherapy regimen were CEAM (lomustin,etoposide,cytarabine and melphalan)for Lymphoma,busulfan and etoposide for AML and melphalan for multiple myeloma patients. **Results.** During this time, 68 HD patients with median age 28 years (range;16-50),67 NHL patients with median age 29 years(range;18-55), 74 MM patients with median age 53 years(range;31-70)and 44 AML patients with median age 27(range;17-51) underwent autologous peripheral blood stem cell transplantation. The median time to platelet count > 20×10^9/L was 15 days (range;9-35). The median time to ANC > 0. 5×10^9/L was 12 days (range;8-27). All patients have engrafted and there were not graft failure in this study group. 100 days transplant related mortality rate was 2. 7% in MM, 2. 9% in HD, 4. 4% in NHL and4. 6% in AML group respectively. **Conclusions**. Our data reflects the important role of HDT followed by HSCT in improvement of outcome for a variety of hematologic malignancies in our center. We concluded high dose therapy rescued with non- cryopreserved auto SCT is safe and effective method that is feasible in our patients.

1872

SECONDARY CANCERS FOLLOWING STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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Background. Hematopoietic stem cell transplantation (HSCT) is an indispensable tool to treat hematologic disorders while secondary cancers may develop as a result of inherent genetic predisposition and high-dose therapy-induced genetic damage. Secondary cancers remain as a devastating late complication following HSCT. We aimed to investigate the frequency and clinical course of secondary malignancies in patients who underwent HSCT and survived more than 100 days. **Methods.** We reviewed the case files of 452 patients (Hematologic malignancy n: 460, Non-malignant hematologic disorders no:9) who underwent allogeneic and autologous stem cell transplantation in our center between 1993 and 2010. One hundred and three patients were excluded from further analysis (Early mortality n:97, insufficient data:6). We recorded type and median time to development of secondary malignancies, primary indications for HSCT and conditioning regimens. **Results.** The eligible 359 patients were subjected to further analysis. Eight patients developed 9 malignancies at a mean of 35. 8 ±3. 8 months (median 24 months, range 4-135 months) following HSCT for a cumulative incidence of 2,2% (Table 1). The mean age at diagnosis of primary disease was 38. 7 ± 13. 1 years while the mean age at diagnosis of secondary malignancies was 41. 6 years. These secondary cancers involved salivary glands (1 mucoepidermoid carcinoma), bone marrow (2 acute myeloid leukemias), brain (primary CNS lymphoma), bladder (1 transitional cell carcinoma), surrenal gland (1 pheochromocytoma), breast (1 invasive ductal carcinoma in situ), peritonial cavity (1 primary peritoneum carcinoma) and thyroid gland (1 papillary carcinoma, follicular variant). All patients with secondary solid cancers were treated successfully whereas patients with secondary hematologic malignancies succumbed to disease progression. **Discussion.** It is a well-known fact that incidences of lymohoma, leukemia, glioblastoma, malignant melanoma, lung cancer and rectal adenodcarcinoma are significantly increased in comparison to sex and age-matched healthy subjects. Risk of developing secondary cancer gradually increases over time. Employment of anti-thymocyte globulin, T-cell depletion, HLA-mismatch, presence of graft versus host disease are found to be related to increased risk of secondary malignancy formation. As a consequence, we recommend that patients be screened for potential secondary cancers. Early diagnosis enables early intervention, thus providing improved survival rates.

Table 1. Demographic and clinical features of patients with secondary malignancies following HSCT.

Patients	Sex/age	HKHN Indication/ Source	Secondary Malignancy	GVHD	Mean time to SM	Management
1	M/42	AML 1.CR/ allo-HSCT	Mucoepidermoid carcinoma	Negative	34 months	Surgery+RT, complete response
2	M/19	Hodgkin's Disease 3.CR/Autologous PBSCT	AML preceded by MDS	Nonapplicable	17 months	Died after Allo-HSCT
3	M/31	AML 1.CR/allo-PBSCT	Primary CNS Lymphoma	Chronic GvHD	4 months	Died, unresponsive to CT+RT
4	M/29	Hodgkin's Disease 2.CR/Autologous PBSCT	AML	Nonapplicable	24 months	Died due to AML
5	M/54	Multiple myeloma partial remission/Autologous PBSCT	Transitional carcinoma of bladder	Nonapplicable	17 months	Surgery, complete response
5	M/54	Multiple myeloma partial remission/Autologous PBSCT	Pheochromocytoma	Nonapplicable	44 months	Surgery, complete response
6	F/47	Multiple myeloma Complete remission/Autologous PBSCT	Invasive ductal carcinoma in situ	Nonapplicable	24 months	Surgery, complete response
7	F/56	Multiple myeloma Complete remission/Autologous PBSCT	Papillary carcinoma, follicular variant	Nonapplicable	23 months	Surgery+ RAI, complete response
8	F/32	Chronic myeloid leukemia/ Chronic Phase	Primary Peritoneum Carcinoma	Nonapplicable	135 months	Surgery and chemotherapy, complete response

1873

POST-TRANSPLANT CYTOMEGALOVIRUS REPLICATION DETECTED BY REAL-TIME PCR IS ASSOCIATED WITH A DECREASED RELAPSE RISK: A JAPANESE SINGLE INSTITUTE STUDY

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Background. Recent publication from Germany reported cytomegalovirus (CMV) replication early after allogeneic stem cell transplantation (allo-SCT) decreased relapse risk in AML patients. In Japan, unlike Western countries, over two-third of adults have been infected with CMV in their childhood. The relevance of CMV replication to relapse might be different. **Aims.** To evaluate the relevance of CMV replication, which was evaluated by using real-time polymerase chain reaction (PCR), to relapse after allo-SCT. **Methods.** We retrospectively analyzed 72 cases with hematological malignancy who underwent allogeneic transplantation between April 2008 and November 2011 in our hospital. CMV replication was monitored weekly. We used PCR to detect CMV replication because pp65 antigenemia is not suitable at the time of low WBC count. We set the cut-off value of CMV positivity as 500copy/ml. Preemptive viral therapy using ganciclovir or foscarnet was initiated to CMV-positive patients. Relapse of malignancy was evaluated by bone marrow examination performed at day 28, 100, and as needed thereafter. As for lymphoma cases, PET-CT was performed when relapse was suspected. This study was approved by institutional review board. **Results.** A total of 53 patients were CMV-positive and 19 were negative. Each CMV-positive/negative group contained 33/14 cases of myeloid malignancy (AML, MDS, CML, MPD) and 9/3 cases of ALL, 8/0 cases of NHL and 3/2 cases of other diseases. There were no significant difference as for patients' sex, age, donor type, donor/recipient CMV serology, disease status, stem cell source, and preparative regimen within both groups. A total of 16 patients relapsed at a median of 126 days□range, 28-755 days□. Although univariate analysis did not show statistical significance, the cumulative incidence of relapse (CIR) at one year tended to be lower in CMV-positive group than CMV-negative one (18. 7% vs. 34. 8%, HR 0. 58; 95% CI 0. 22-1. 50; *P* =0. 26). In the each subgroup of patients with myeloid malignancy (n=47), advanced disease status (n=45), surviving free of relapse at day100 (n=25), CIR also tended to be lower in CMV-positive group. Univariate analysis in all patients also indicated that myeloablative preparative regimen and non-advanced disease status had a tendency to decrease CIR (*P* <0. 1). Multivariate analysis of

CIR in all patients which included preparative regimen, disease status and CMV positivity as covariates showed that CMV positivity was independently correlated with reduced relapse risk (HR 0. 388; 95% CI 0. 15-0. 97; *P* =0. 045). In this study, most of CMV-positive patients were in advanced disease status, which might be the reason why univariate analysis of CMV positivity did not show significant result. **Conclusions.** This is the first report that revealed CMV replication, demonstrated by real-time PCR, is an independent factor associated with reduced relapse risk after allo-SCT.

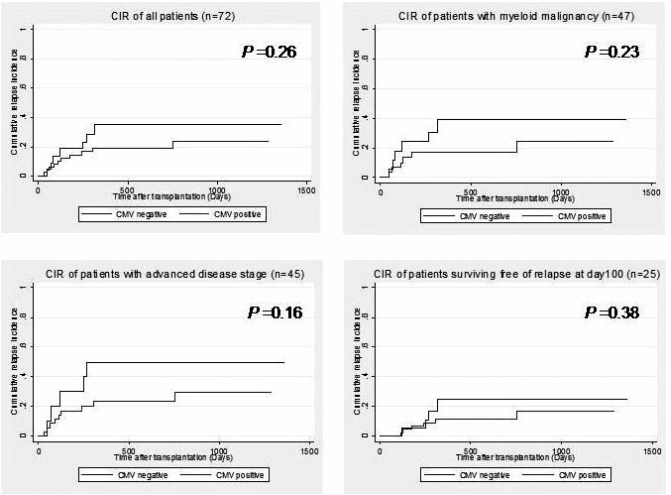

Figure 1. Cumlative relapse incidence [CIR] of all patients and subgroups.

1874

LOW COUNTS OF CD4-CD8- T-CELLS DURING THE FIRST WEEKS AFTER UMBILICAL CORD BLOOD TRANSPLANT

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Background. CD3+CD4-CD8- T-cells can suppress alloimmune responses. However, the mechanisms by which these cells regulate immune responses and their role in the immune recovery after hematopoietic stem cell transplantation (HSCT) remain unknown. **Aims.** To compare the distribution of different subsets of T-cells and NK cells after unrelated allogeneic transplant in patients receiving umbilical cord blood (UCB), bone marrow (BM), or peripheral blood stem cells (PBSC). **Methods.** T-cells (CD4+/CD8+/CD4-8-/CD4+8+), and NK cells subsets (lineage negative and 56++16-/56+16++) were quantified by multiparametric flow cytometry at 6 sequential time points (at engraftment, and at days 3, 7, 14, 21 and 60 after engraftment). Overall, 34 patients (19 male; median age 13y, range 1-63y) receiving a UCB (n=15), BM (n=14) or PBSC (n=5) unrelated HSCT were studied. The most common diagnosis was acute leukemia (ALL, 12 cases; AML, 10; CML, 5; aplastic anemia/MDS, 6; Hodgkin lymphoma, 1; SCID, 1). Most patients received myeloablative conditioning (MAC) regimens (73%). Antithymocyte globulin (ATG) was used in 38% and total body irradiation (TBI) in 41% of cases. Median time to neutrophil engraftment was 18 days (range: 12-45). Median follow up time was 6 months. **Results.** As compared to BM/PBSC, UCB was associated with a delayed neutrophil engraftment (28 days vs. 17 days; p=. 01), and a trend to lower counts of all T-cell in the first 3 weeks. At day 14 after engraftment, the median number of total T CD3+ cells was 149/uL for UCB vs. 828/uL for BM/PBSC recipients (p=. 0004). The median number of CD4-CD8- T-cell was significantly lower in UCB recipients as compared to BM and PBSC at all times during the first 3 weeks (median 2/uL vs. 22/uL at day 3, p=. 001; 2/uL vs. 13/uL at day 7, p=. 003; 3/uL vs. 27/uL at day 14, p=. 005; and 3/uL vs. 45/uL at day 21 after engraftment, p=. 04). At day 60, Total T and CD4- 8- cells counts were comparable between UCB and BM/PBSC recipients (11/uL vs. 30/uL, p=. 13). No significant differences were observed between both groups as regards the distribution of NK cells subsets at any time. There was no significant influence of MAC, ATG or TBI on the differences in T-cell counts observed between the two groups. **Conclusions.** UCB recipients have lower counts of CD4-/CD8- T-cells during the first weeks after HSCT as compared to BM or PBSC recipients. The impact of this finding on the transplant outcome remains to be determined.

1875

RISK-ADAPTED DONOR LYMPHOCYTE INFUSION TO PREVENT RELAPSE IS INDICATED IN ALL PATIENTS WITHOUT GVHD AFTER T CELL DEPLETED MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION FOR AML

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Background. The aim of allogeneic stem cell transplantation (HSCT) for acute myeloid leukemia (AML) is curation by a graft-versus-leukemia (GVL) effect mediated by alloreactive donor T-cells. However, donor T-cells may also induce graft-versus-host-disease (GVHD) after HSCT. T-cell depletion (TCD) reduces GVHD but increases relapse rates. Relapse may be prevented by pre-emptive post-HSCT donor lymphocyte infusion (DLI) by reintroducing the GVL-effect. Early DLI may be effective but can cause severe GVHD, whereas late DLI will cause limited GVHD but early relapses may occur. Patients with full-donor chimerism (FD) have been reported to have a low incidence of relapse, whereas patients with mixed-chimerism (MC) are considered to have a high risk of relapse. **Aims.** In this study, we investigated whether in AML/MDS patients following myeloablative (MA) conditioning DLI could be postponed to 6 months after TCD-HSCT. In addition, we investigated whether in patients with FD chimerism pre-emptive DLI could be omitted. **Methods.** 39 patients underwent MA TCD-HSCT for AML (n=34), high risk MDS (n=4, median IPSS 2) or CMMol (n=1) between 2002 and 2007. Of the 34 AML patients, 7 had a very-poor-risk monosomal-karyotype (VPR), and 25 were poor-risk (other unfavorable cytogenetics/molecular abnormalities and/or transplantation in CR2). Conditioning was performed with cyclophosphamide-TBI, TCD with CD34+cell-selection (n=6) or alemtuzumab *in vitro* (n=33). Six months after HSCT patients were evaluated for active GVHD, i. e. requiring systemic immune-suppression. In the absence of GVHD chimerism was measured. In case of MC, patients with a related donor (n=32) were scheduled to receive 3. 0x10^6 CD3+cells/kg and patients with an unrelated donor (n=7) received 1. 5x10^6 CD3+cells/kg. Primary endpoint was relapse, secondary endpoint was non-relapse mortality. **Results.** After HSCT, only 8% of patients developed grade 3-4 acute GVHD. During the first 6 months, 4 patients (10%) died of non-relapse mortality (NRM) and 8 patients (21%) relapsed (4 poor-risk and 4 VPR). Six of the remaining 27 patients had active GVHD at 6 months, and were not eligible for DLI. Twelve patients were eligible for DLI because of MC. Three of these 12 patients relapsed before DLI was given (1 poor-risk and 2 VPR). Median time to DLI was 210 days (range 143-315 days). After DLI, 2 patients suffered a relapse (1 poor-risk, 1 MDS) and 2 died of NRM. Nine patients without GVHD were FD and therefore initially did not receive pre-emptive DLI. Four of these 9 patients relapsed during follow-up (all poor-risk). Another 4 patients converted to MC and were treated with DLI, of whom none relapsed. After DLI, only one patient developed >grade 1 acute GVHD. Relapses were observed in patients without conversion in chimerism only. **Conclusions.** Relapses frequently occurred within the first 6 months after myeloablative TCD-HSCT in patients with very-poor-risk AML. These patients should receive DLI early after transplantation. Furthermore, the high relapse rate in patients with FD chimerism without GVHD indicates a lack of GVL-effect in these patients, indicating that these patients should also be treated with pre-emptive DLI.

1876

EFFICACY OF APREPITANT IN PREVENTING HIGH-DOSE MELPHALAN-INDUCED NAUSEA AND VOMITING IN PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. High-dose melphalan has been commonly used alone or in combination with other agents for the conditioning of hematopoietic stem cell transplantation (HSCT). High-dose melphalan has a potential to cause both acute and delayed unacceptable emesis. Although melphalan is categorized as a moderately emetogenic agent, there is little data regarding the level of emetogenecity of its high-dose administration. Therefore, an optimal antiemetic regimen has yet to be established in this setting. **Aims.** The primary aim of this study is to evaluate the antiemetic efficacy of aprepitant in patients receiving

high-dose melphalan as a component of conditioning of HSCT. **Patients and Methods.** Fifty-three patients who underwent allogeneic HSCT at Keio University Hospital (Tokyo, Japan) after being conditioned with high-dose melphalan (70 mg/m^2/d for 2 days) in combination with fludarabine (125 mg/m^2) were retrospectively evaluated. Data were collected from the institutional data bases and the medical records. Until the introduction of aprepitant, 40 patients received only the standard antiemetic regimen consisting of ondansetron 4 mg every 12 hours and iv steroid on the 2 days of melphalan administration (Control). Thirteen patients received aprepitant orally for 5 days (125 mg followed by 80mg for 4 days) in addition to the standard regimen (Aprepitant group). Incidence and number of vomiting from the first day of melphalan administration through 10 days after transplantation were compared between the two groups. **Results.** There were no significant differences in age, sex, diagnosis and source of stem cells between the two groups. The complete protection (no emesis/vomiting) rate was 92% (12 of 13 patients) in Aprepitant group which was significantly higher than 33% (13 of 40) in the Control (P<0. 001). One patient in Aprepitant group vomited only once, while median number of vomiting during the observation period was 2 (range: 0-37) in the Control (P<0. 001). There were no significant side effects considered due to aprepitant. **Conclusions.** These results strongly suggest that addition of aprepitant could be superior to the standard antiemetic regimen with ondansetron in preventing high-dose melphalan-induced nausea and vomiting.

1877

HIGH RATE OF EBV REPLICATION FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION - PRELIMINARY DATA OF A ROUTINE MONITORING POLICY

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Epstein-Barr Virus (EBV) reactivation is an important complication following allogeneic stem cell transplantation (HSCT) with a wide range of clinical presentations, ranging from asymptomatic replication to aggressive post-transplant lymphoproliferative disorder (PTLD). While the latter clearly requires systemic treatment, the value of asymptomatic EBV replication remains unclear. Furthermore, while monitoring of high-risk patients is recommended, routine monitoring of all patients is not always performed. Following 2 fatal cases of highly aggressive PTLD, we have installed routine monitoring following HSCT in our transplant unit. Here we present preliminary data of this monitoring policy. We performed a retrospective analysis of the first 30 patients who received an allogeneic transplant after implementation of routine monitoring. EBV load was measured in whole blood using a real-time polymerase-chain-reaction assay. Monitoring was performed weekly during the first month posttransplant and at least monthly thereafter. In case of EBV replication, monitoring frequency was increased, and patients were regularly examined for signs of PTLD. The decision to administer preemptive treatment in the absence of PTLD was made at the discretion of the treating physician and was based on clinical condition as well as the absolute level of EBV load. Patients with biopsy proven PTLD were treated with rituximab. Patient characteristics are shown in Table 1.

Table 1.

	n=30
Disease	
Acute myeloid leukemia	7
Myelodysplastic syndrome	6
Acute lymphatic leukemia	4
Multiple myeloma	4
Lymphoma	6
Myeloproliferative neoplasm	1
Aplastic anemia	2
Donor	
Identical sibling donor	10
Matched unrelated donor	15
Mismatched unrelated donor (9/10)	3
Haploidentical donor	2
Stem cell source	
Peripheral blood stem cells	26
Bone marrow	4
Conditioning	
Myeloablative	19
Reduced intensity	11
Containing ATG	16

Patients were transplanted between May and November 2011. After a median follow up of 4. 5 months (1-6), 17 (57%) patients showed EBV replication posttransplant. The median time to first positive result was 28 days (5-146). The median EBV peak level was 14'237 (range 541-3'257'101). 16 patients showed no clinical signs of PTLD, while one patient had biopsy proven PTLD. This patient and 4 others were treated with 1 to 5 doses of rituximab. In the patients without PTLD, the decision to treat was based on poor clinical condition in two patients with rapidly progressing hepatopathy and fulminant acute respiratory distress syndrome (ARDS), respectively, though it remained unclear whether the poor condition was associated with EBV replication. One patient was treated due to intense immunosuppression following treatment for severe GvHD, and finally one patient was treated due to rapidly rising EBV levels following anti-thymocyte globuline (ATG). Of the 30 patients, 6 died due to relapse (n=2), GvHD (n=1), pulmonary venoocclusive disease (n=1), sepsis (n=1) and ARDS (n=1). Of the deceased patients, 4 had had EBV replication, but no deaths were deemed to be attributable to EBV. Small patient numbers precluded extensive analysis of risk factors for EBV replication. However we observed a strong correlation of EBV replication with ATG-containing conditioning, a well known risk factor, with EBV replication in 14 of 16 patients (88%) who received ATG compared to 3 of 14 (21%) patients who did not receive ATG (p=0. 001). In summary, in this preliminary analysis of routine EBV monitoring, we observed a high rate of EBV replication in 57% of patients, but only one case of PTLD, with ATG being an important risk factor. However, only a randomized trial can show whether EBV monitoring and preemptive treatment strategies lead to decreased morbidity and mortality.

1878

COMPARATIVE STUDY OF LARGE VOLUME APHERESIS EFFICACY IN MULTIPLE MYELOMA AND LYMPHOMA

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Background and Objectives. In the mobilization of hematopoietic progenitor cells from the bone marrow, apheresis has been described to have a mobilizing effect, so that the processing of more than three volume blood volumes increase the yield of peripheral blood progenitor cells (PBPCs). For this reason large volumes apheresis (LVA) are processed in some centres, independently of the pathology. **Aims.** The aim of this study is to establish if harvest efficiency of LVA varies according to the diagnosis. Multiple myeloma (MM) and Lymphoma were studied because they are the most frequent indication of autologous transplant. Materials and **Methods.** LVA performed in the past ten years in our hospital are reported. PBPCs were obtained using a continuous blood cell separator (Fenwal CS3000; Baxter, Deerfield, IL). Four times the calculated blood volume was processed in each apheresis. Mononuclear cells (MNC), CD34+ cells and clonogenic CFU-GM, BFU-E and CFU-GMM measures were performed: in peripheral blood pre-apheresis, in the product obtained after the first two volumes (1st bag) and after the second two volumes (2nd bag). Total pre-apheresis values (TPAV) were calculated according to the estimated total blood volume of the patient. Recovery of these variables in each one of the two bags was calculated as percentage of TPAV. Student-t test was used for comparison between MM and Lymphoma group results. **Results.** A total of 150 apheresis, performed to 61 patients with MM, and 184 performed to 68 patients with Lymphoma (50NHL and 18 HD) were included in the study. Results are shown in the following Table 1.

Table 1.

Variables	Lymphoma (n=184)	Multiple Myeloma (n=150)	p
CD34+ cells x10^9/L Pre-apheresis	10.545±1.73	22.05±2.61	<0.001
CD34+ cells Recovery 1st bag (%)	111.44±10.14	100.43±6.20	0.378
CD34+ cells Recovery 2nd bag (%)	133.03±8.05	134.36±10.99	0.921
MNC Recovery 1st bag (%)	391.80±224.88	155.63±15.28	0.356
MNC Recovery 2nd bag (%)	267.27±57.71	371.95±85.45	0.295
CFU-GM Recovery 1st bag (%)	308.47±51.26	378.34±60.83	0.377
CFU-GM Recovery 2nd bag (%)	383.87±83.62	340.42±56.28	0.681
BFU-E Recovery 1st bag (%)	272.13±54.67	204.51±42.44	0.323
BFU-E Recovery 2nd bag (%)	321.02±93.86	222.04±34.69	0.296
CFU-GEMM Recovery 1st bag (%)	317.51±60.01	332.99±81.60	0.880
CFU-GEMM Recovery 2nd bag (%)	562.53±194.47	361.82±62.67	0.312
Progenitor cells recovery after the first two blood volumes processed (1st bag) and after the second two volumes (2nd bag), expressed as mean± standard error of the mean			

CD34+ cells pre-apheresis were significantly higher in MM (22. 05 CD34+ cells

x10^9/L) than in lymphoma (10. 54 CD34+ cells x10^9/L), p < 0. 001. CD34+ cells recoveries were significantly higher in the second bags compared with the first ones in both pathologies (p < 0. 001). However, no significant difference in LVA efficacy in recovery of CD34, MNC and colony forming units (CFU-GM, BFU-E and CFU-GEMM) was found between MM and lymphoma. **Conclusions.** LVA is equally effective in these patients, independently of the pathology; more than 100% of the TPA values are recovered in each one of the two bags. The 2nd bag recovery (equal or higher than the 1st) corroborates the mobilizing role of the apheresis procedure.

1879

AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION IS A HIGHLY EFFECTIVE SECOND LINE OF TREATMENT FOR PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) remains one of the most frequently seen non-Hodgkin lymphoma (NHL) with an aggressive disease course. It estimates that only 40-50% of patients (pts) may be cured with chemo - and radiotherapy; the remaining pts subset remains partially chemosensitive or resistant. High dose chemotherapy (HDT) followed by autologous haematopoietic stem cell transplantation (AHSCT) is a method of choice for the pts who didn't achieve complete remission (CR) after R-CHOP or CHOP treatment. We present 80 pts with DLBCL who were underwent AHSCT between January 1999 and April 2011 in our Department. There were 47 male and 33 female, with a median age of 52. (range 18-68 yrs). Ann Arbor staging at diagnosis was as follows: II- (n=11), III- (n=17), IV- (n=32); 48 of pts manifested B-symptoms. 50 of pts had an aged-adjusted IPI 2 or 3, 8 pts - IPI 4. Clinical manifestation at diagnosis included: hepatomegaly (n=16), splenomegaly (n=19), enlargement of the lymph nodes (n=39), bone marrow infiltration (n=7), lung infiltrates (n=5), digestive system involvement (n=9), CNS (n=4), tonsils (n=3). Initially, all were treated CHOP but 65 of them received chemotherapy with rituximab and achieved partial response (PR) which was defined as the reduction of measurable disease by ≥50% without the appearance of any new lesions. Patients with PR proceeded to high dose chemotherapy (HDT) followed by AHSCT. Stem cells were collected from peripheral blood after IVE chemotherapy (IVE - ifosfamide 3g/m2 iv in 1-3d, etoposide, epirubicine 50mg iv in1d) in 67 patients, in 9 with other treatment and subsequent administration of granulocyte-colony stimulating factor (G-CSF) at a dose of 10ug/kg/d, starting from +5 day of chemotherapy till the last day of collection. G-CSF alone (10ug/kg/d) was used in 4 remaining patients. Collections were performed using Optia Spectra. All patients collected the sufficient number of CD34+ cells for AHSCT procedure. Conditioning regimens preceeding AHSCT consisted of CBV in 73 cases, BEAM in 6 and LACE in one. A median number of transplanted CD34+ cells was 3,97 (1. 25 - 35. 76x10^6/kg). All patient successfully engrafted. Hematopoietic recovery was as following: WBC count > 1,0x10^9/L after median of 12 days (range 8-16 days),ANC> 0,5x10^9/L after median of 14 days (range 8-17 days) and platelet count >20x10^9/L after median of 14 days (range 7-21 days). None of pts die due to AHSCT (TRM 0%). The major complications after AHSCT were rare and included: bacterial infections of the respiratory tract (n=15), viral infections (n=10), oral mucositis (n=9). 145 months' disease free survival (DSF) was estimated to be 86% with a 145 months' overall survival of 88%. 69 patients achieved CR after AHSCT (86,3%). Six pts underwent second AHSCT and 4 of them achieved CR. At the last contact, 75 pts are alive with a median follow-up period of 56 months (range 3-145). 5 patients died due to disease progression. HDT followed by AHSCT seems to be highly effective and safe procedure for DLBCL patients.

1880

THE SECOND AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION IS A HIGHLY EFFECTIVE TREATMENT FOR MULTIPLE MYELOMA PATIENTS WHO DIDN'T ACHIEVE COMPLETE REMISSION AFTER THE FIRST AHSCT

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High dose chemotherapy (HDT) followed by autologous haematopoietic stem cell transplantation (AHSCT) is a standard method of treatment for the patients (pts) with multiple myeloma (MM) under 70 year old. This therapy prolongs disease free survival and time to relapse. In our Department 298 pts with MM were underwent AHSCT till April 2011, what was 21% of whole 1405 haematopoietic stem cell tansplantation. 56 pts who didn't achieve complete remission(CR) after the first AHSCT were undergone the second AHSCT. Stem cells were collected from peripheral blood after IVE (ifosfamide 3g/m2 iv in 1-3d, etoposide, epirubicine 50mg iv in1d) or Endoxan 2,0-4,0g/m^2 chemotherapy and subsequent administration of granulocyte-colony stimulating factor (G-CSF) at a dose of 10ug/kg/d, starting from +5 day of chemotherapy till the last day of collection. Collections were performed using Optia Spectra. The second transplantation were underwent in VGPR (33%), PR(n= 56%), SD (11%). Tandem transplantation was performed for 90 days from the first AHCT in 8 pts, and the other underwent the second AHSCT within the next 3 months. The conditioning regiments preceeding AHSCT in all pts consisted of Melfalan 140-200mg/m^2. The median number of transplanted CD34+ cells was 4,6 (1. 8 - 16,7x10^6/kg). All patient successfully engrafted. Hematopoietic recovery was as following: WBC count > 1,0x10^9/L after median of 14 days (range 12-20 days) and platelet count >50x10^9/L after median of 15 days (range 12-21 days). The major complication after AHSCT was mucositis which was observed in 50%pts (n=28). None of pts died due to AHSCT (TRM 0%). 10 years' overall survival for tandem AHSCT estimated 52% in comparison to37% for single AHSCT (p=0,03). The tandem AHSCT procedure seems to be safety and highly effective therapy for prolongation overall survival in myeloma multiple patients, who didn't achieved CR after first autologous transplantation.

1881

EFFICACY OF SUPLATAST TOSILATE AND THE MOLECULAR MECHANISM OF PRURITUS IN POST-TRANSPLANT CHILDREN

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Background. Patients who undergo stem cell transplantation often develop skin pruritus. For these patients, we have tried various drugs including antihistamines and anti-allergic medications, but they have shown poor efficacy. Suplatast tosilate has recently been developed as a unique treatment that selectively inhibits the production of Th2-type cytokines, such as IL4 and IL5, which reduces the production of IgE and the infiltration of eosinophils into tissue. To date, suplatast tosilate has been licensed only in Japan and authorized exclusively for asthma and atopic dermatitis. Because of the specificity of its pharmacological action, we performed a pilot study of suplatast tosilate treatment with 7 post-transplant children with pruritus. **Aims.** To elucidate the clinical efficacy of suplatast tosilate for post-transplant pruritus and to retrospectively identify biomarkers or factors associated with the outcome of this treatment. **Methods.** Seven patients who developed pruritus after stem cell transplantation (5 boys and 2 girls aged 8 months to 12 years) received suplatast tosilate (6-8 mg/kg/day). The clinical response was compared before (point 1) and at 7 days after the treatment (point 2). In addition, the lymphocyte count, Th1/Th2-type lymphocyte fraction, eosinophil count, and the level of Th2-type cytokines (IL4 and IL5) in the peripheral blood were measured at both points. **Result.** Itching disappeared completely within 7 days after treatment in 3 of 7 patients (UPN1, UPN2, UPN3). All 3 patients who achieved a clinical response presented with pruritus within 100 days after transplantation, and 2 patients presented with acute graft *vs* host disease (GVHD). In these 2 patients, the level of IL5 decreased after treatment (10. 3 pg/mL at point 1 and < 3. 9 pg/mL at point 2 in UPN1, 31. 2 pg/mL at point 1 and < 3. 9 pg/mL at point 2 in UPN3); whereas the Th1/Th2 ratio increased (2. 3 at point 1 and 3. 8 at point 2 in UPN1, 1. 8 at point 1 and 14. 2 at point 2 in UPN3). In contrast, the total lymphocyte count, eosinophil count, and the levels of IL4 and IgE did not change significantly. **Conclusions.** Suplatast tosilate may have a promising effect in patients who present with intractable pruritus with acute GVHD and a high level of IL5. The observation that the proportion of Th2-type lymphocytes and the level of IL5 decreased in 2 patients who achieved a complete response suggests that the abnormal T cell profile in the early stage after transplantation may be involved in the pathophysiology of pruritus. Although such pruritus is often neglected, the use of this drug may obviate the additional use of steroids to suppress immune reconstitution and improve patients' quality of life. The present results are encouraging for children with post-transplant pruritus, suggesting a large prospective study is warranted to better determine the efficacy of this treatment.

1882

SUCCESSFUL STEM CELL TRANSPLANTATION IN HOMOZYGOUS ALPHA-THALASSEMIA 1: THE FIRST CASE IN ASIA

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Homozygouse alpha-thalassemia 1 is the most severe thalassemia disease. This group of patients presents with hydrops fetalis and always dies before or shortly after birth. We reported successful stem cell transplantation in homozygouse

alpha-thalassemia 1. A Thai girl was born at 32 weeks of gestational age (birth weight 1,300 gm) with hydrops fetalis by cesarean section. Her mother was 33 years old came to our hospital because of toxemia of pregnancy and prenatal ultrasonography showed hydrops fetalis with massive ascites. Her hemoglobin at birth was 6. 8 g/dl. She was intubated, abdominal, pleural paracentesis and total blood exchange transfusion immediately after birth. Her hemoglobin typing before total blood exchange transfusion was 100% of hemoglobin Bart's and DNA analysis showed homozygouse alpha-thalassemia 1 (South East Asian deletion). After she was stable from preterm complications, she was started on hypertransfusion every 3-4 weeks and kept pre-transfusion hemoglobin at 9-10 g/dl since the age of 3 months. She has 6/6 HLA matched with her older sister. She was underwent stem cell transplantation at the age of 1 year and body weight of 9. 6 kg. The conditioning regimen consisted of busulfan 1. 2 mg/kg/dose every 6 hours intravenously for 4 days and cyclophosphamide 50 mg/kg/day for 4 days. GVHD prophylaxis was cyclosporine and short course metrotrexate. The stem cell source was bone marrow and total CD 34 counts was 9. 41x10^6cells/kg. Her transplantation complications were febrile neutropenia and mucositis. She had WBC engraftment at day+17 and DNA showed full donor chimerism at day+25. On day+112, DNA showed mixed chimerism with 41% of recipient's DNA but hemoglobin was still stable. Cyclosporine was stopped and she received donor lymphocyte transfusion (DLI) at the dose 1x10^7cells/kg of CD3 from her sister every 2 weeks. After 4 times of DLI, she could achieve full donor chimerism. Now, she is 4 years old with post transplantation for 3 years and her DNA is still full donor chimerism. Her hemoglobin is 13 g/dl, normal hemoglobin typing and she never receives blood transfusion for almost 2. 5 years. Her developmental milestones are up to her age. **Conclusions.** Homozygouse alpha-thalassemia 1 can be cured with stem cell transplantation. Preterm delivery with immediate total blood exchange transfusion, hypertransfusion and proceed to stem cell transplantation is possible treatment strategy to cure from this fatal disease. From our knowledge, this is the first successful stem cell transplantation in homozygouse alpha-thalassemia 1 in Asia.

1883

ALLOGENEIC STEM-CELL TRANSPLANTATION FOR THERAPY-RELATED ACUTE MYELOID LEUKEMIA (T-AML) AND MYELODISPLASTIC SYNDROME (T-MSD): A SINGLE CENTER EXPERIENCE.

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Background. WHO 2008 defined Therapy-related myeloid neoplasm category includes acute myeloid leukemia (t-AML), myelodisplastic syndrome (t-MDS) and myelodisplastic/myeloprolipherative neoplasms (t-MDS/MPN) occurring after chemo- and/or radiotherapy administered to treat a previous neoplasms or a non neoplastic disorders. They account for 10-20% of all cases of AML, MDS and MDS/MPN. The prognosis of these patients is worse than their de novo counterpart, with a 5-year overall survival (OS) of less than 10%. **Aims.** Our retroprospective study has the purpose to analyse the role of allogeneic stem cell transplantation (aSCT) in this setting of diseases. **Methods.** Our analysis includes 17 patients (10 female,7 male) with median age of 53 years (range 29 - 64), observed at ourInstitute of Hematology, receiving aSCT between September 1999 and January 2012. All patients were treated for a primary neoplasm with chemo- and/or immuno- and/or radiotherapy (median number of 2 lines of therapy). Primary neoplasms consisted of 7 hematologic malignancies (2 B-CLL/SLL, 1 low-grade NHL, 3 DLBCL, 1 HL) and 10 solid tumors mainly breast cancer (7/10). The median time between the first treatment to tAML/tMDS/MPN was 33 months (range 12-144 months). Seven patients had AML, 9 MDS and 1 Ph+ ALL. Karyotype was available for 14/17 patients: 2 abnormalities of chromosome 7, 4 normal karyotype, 2 complex karyotype, 1 patient with 16 monosomy/+13, 1 del(11)(q14;q23), 1 t(9;22), 1 t(9;16), 1 del(20) and 1 was hypodiploid. Thirteen/17 patients received an induction treatment before aSCT: 10 patients chemotherapy, 2 5-azacitidine, 1 both treatments. Five/17 patients (29%) received conditioning regimen in complete remission (CR). A myeloablative conditioning (MAC) was used in 29. 5% of patients (BuCy2), while a nonmyeloablative transplantation was preferred for the remaining 70. 5% (8 patients FluTTMel, 4 BuFlu). GVHD prophylaxis included cyclosporine and MMF in more than 50% of the patients. All 9 patients receiving matched unrelated donor (MUD) aSCT were treated with additional immunosuppressive therapy. G-CSF mobilized peripheral blood hematopoietic stem cells were used in all patients. Median HCT-CI was 3 (range 0-3). **Results.** Three patients died within one month after aSCT (17. 7%). Early deaths were attributable to infectious complications and acute GVHD. Two patients are too early to be evaluated. Among 12 surviving at least 100 days after aSCT we reached a response rate of 83% (10/12). Two patients relapsed after 4 and 12 months respectively after aSCT and both of them died from their disease at 3 and 5 months respectively. At a median follow-up of 27 months (range 4-136), 6 patient are alive in cCR (35%). **Conclusions.** As reported in the literature the only curative option in these patients is represented by aSCT. We report data from a small series referring to our centre. All patients eligible for aSCT underwent eventually transplantation. Despite an high TRM, high risk cytogenetic and disease status at transplantation (71% with active disease), 6 patients (35%) are in cCR at a median FUP of 27 months. We confirm that aSCT is a feasible approach although this subset of patients per se has a high HCT-CI.

1884

THE COMBINATION OF TACROLIMUS AND SIROLIMUS IN HIGH-RISK ALLOGENEIC HSCT WITH BU-FLU CONDITIONING

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Background. GVHD remains a major cause of morbidity and mortality after allogeneic HSCT. The combination of sirolimus (Sir) and tacrolimus (Tac) has resulted in a low incidence of acute GVHD and reduced transplant-related toxicity in several studies. We sought to confirm the efficacy of sirolimus in combination with Tac for GVHD prophylaxis in unrelated HSCT. **Methods.** 65 patients received peripheral blood stem cells from unrelated donor (n=60) or mismatched sibling donor (n=5) after conditioning regimen consisting of busulfan and fludarabine. And patients were treated with 3 different regimens for GVHD prophylaxis: CsA/MTX (n=20), Tac/MTX (n=21), and Tac/Sir (n=24). **Results.** The incidence of grade II-IV acute GVHD in Tac/Sir group was lower than other groups (25% for Tac/Sir vs 45% for CsA/MTX, 43% for Tac/MTX). The day-100 NRM in Tac/Sir group was also lower than other groups. The VOD of liver was developed in five cases (one with CsA/MTX, two with Tac/MTX, and two with Tac/Sir). Only one case of thrombotic microangiopathy developed in the Tac/Sir group. The incidence of CMV or EBV reactivation in Tac/Sir group was higher than other groups. To evaluate immunologic recovery around D+100 day, there was no significant difference in immune reconstitution. **Conclusions.** The combination of tacrolimus and sirolimus is well tolerated and may be helpful in deceasing the risk of acute GVHD and NRM in case of high risk allogeneic HSCT. However, we need more efforts to find ways to differentiated strategy according to the risk of acute GVHD because of its adverse effects.

1885

WITHDRAWN

1886

FOUR CASES OF PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY AFTER STEM CELL TRANSPLANTATION

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Background. Progressive multifocal leukoencephalopathy (PML) is a rare subacute demyelinating disease of the central nervous system (CNS) caused by polyomavirus (JCV/BKV). In immunocompromised patients it is caused by reactivation of latent infection. Altought in the context of stem cell transplantation (SCT) PML is a rare complication, it must be ruled out in transplanted patients (autologous or allogeneic) with neurological symptoms. Limited data ara available on caracteristics of PML in this setting. There is currently no effective therapy to control Polyomavirus, and the prognosis is dismal in most cases. **Aims.** To describe a series of 4 patients transplanted in our institution with neurological symptoms who were diagnosed with PML. **Results.** The characteristics of the patients are listed in Table 1. Four patients who underwent tandem autologous (1) and allogenic SCT (2,3,4) between 2004 and 2011, were diagnosed with PML. All patients had controlled hematological disease (1 RP, 2 VGPR, 3

CR, 4 RC) at the time of PML diagnosis. All patients has been heavely treated and 2 patients were receiving inmunosuppression (IS) at the time of PML diagnosis. Clinical manifestations have been heterogeneous. Only in 2 patients the lesions described on MRI were consistent with PML (1,3). PCR in CSF was diagnostic in all four cases: JCV (1,3,4) and BKV (2), and tests for other causes, including other viruses, bacteria, fungi and tuberculosis, were negative. IS tapering and treatment with mefloquine/citalopram[1] were started, however all patients in our series progressed and died in a median of 2 months after the diagnosis of PML. **Conclusions.** PML in the context of allogeneic or autologous transplantation should be suspected in patients with neurological symptoms,especially in those with poor immune reconstitution, such as alternative donor transplant recipients. Patients in our series had received several lines of therapy, including previous autologous SCT in 3. The clinical presentation of PML can be very heterogeneous, and not always the radiological pattern is consistent with the suspicion of PML, therefore CSF PCR for Polyomavirus should be included in the diagnostic work-up in this setting.

Reference

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Table 1.

N°	Sex/age	Diagnosis	Previous Therapy (n° lines)	Stem cell source/donor	Conditioning/ IS	Onset after Transplant (months)	Neurological manifestations	Neuro-imaging MRI	CSF PCR	Treatment	Outcome
1	F/42	Hystiocitic sarcoma, 2008	5	Autologous SCT TANDEM	-BEAM (1°) - Iv Bu/Flu(2°)	3	diplopia, dysmetria and gait disturbances	white matter right cerebellar, thalamus and basal ganglia lesions	JCV	Mefloquine+ citalopram	Dead after 2 months
2	M/55	MM IgA lambda IIa relapse after 2 autologous sct 1998	6	BM haploidentical	RIC Cyclosporine+MMF+ CTX	13	Right Hemiparesis	cerebellar and hemisferic subacute ischemic lesions	BKV	IS tapering	Dead after 1 months
3	M/59	MM IgA kappa plasmablastic, relapse after 2 autologous stc 2004	5	PB matched unrelated	RIC+ Bortezomib Cyclosporine+ MTX	10	Confusion, altered mental status	frontal white matter lesions	JCV	Mefloquine+ Seropram	Dead after 1 months
4	M/53	MDS AREB 2002 LMA-M6 2007	1	Cord blood	MA Cyclosporine+ MMF + ATG	53	Disartria, impaired Fine movement	diffuse alteration of brainstem, thalamus, White matter lesions cerebellar and both hemisferic	JCV	Mefloquine+ Citalopram	Dead after 3 months

SCT: stem cell transplantation, PB: Peripheral blood, CTX:cyclophosphamide. MMF: Micophenolate, MTX: Methotrexate, MA: myeloablative RIC: reduce intensity conditioning

ATG: Anti-thymocyte globuline. MRI: magnetic resonance imaging.

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SAFETY AND QUALITY OF HAEMATOPOIETIC STEM CELLS FOR TRANSPLANTATION

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Background. It is known that biological and functional properties of haematopoietic stem cells (HSCs), which are commonly used in treatment of malignant and non-malignant diseases, play a crucial role in influencing the outcome of the transplant, considered as achievement of a rapid and durable granulocytes-platelets recovery. Because this is particularly related to CD34+ cells content of the graft, an important aspect is to ensure an adequate cryopreservation and storage of autologous HSCs to guarantee their integrity until the transplant. **Aims.** Based on this consideration, we standardized an internal protocol of quality controls to evaluate the main biological properties of the graft and to obtain its validation. At the same time we could predict, approximately, just at collection time, the real CD34+ cells count expected at the transplant. **Methods.** The most common parameters recognized as indicators of quality are total nucleated cells (TNC), mononuclear cells (MNC), cell viability, CD34+ cells count, colony forming unit-Granulocyte Macrophage (CFU-GM), microbial and minimal residual disease (MRD) contamination. In 2011, we analyzed 63 cryopreserved autologous peripheral blood stem cells (PBSCs) collections. The timing of our protocol is reported as follows. Collection and processing: PBSCs, collected using a leukapheresis cell separator (Fresenius COM-TEC), were assessed for TNC, MNC, CD34+ cells (ISHAGE Protocol, FacsCalibur, BD), cell viability (7-AAD), CFU-GM, microbial and MRD contamination. At this step we calculated, using MNC% value, the probable CD34+ performance. After 30 days of cryopreservation and storage in liquid N2: revaluation of cell viability (trypan blue) on aliquots stored together with PBSCs unit and evaluation of approximate CD34+ count expected at release. This test is also performed immediately before the transplant to verify the effective performance of the cellular product. At transplant: after thawing and washing, we evaluated TNC, MNC, CD34+, cell viability (7-AAD, trypan blue) and microbial contamination to definitively establish the quality of the graft before releasing. **Results.** The data are shown as mean value. At collection: TNC 180x10E8 (r. 130-210); MNC 67,9% (r. 40-87), CD34+ 4x10E6/Kg (r. 0,54-7,4), CD34+ performance 2,7x10E6/Kg (r. 0,38-5,2). After 30 days of cryopreservation: cell viability 67% (r. 39-82), CD34+ performance 2,6x10E6/Kg (r. 0,33-5,1). At transplant: TNC 158x10E8 (r. 114-184); cell viability 68,8% (r. 42-92), CD34+ 2,8x10E6/Kg (r. 0,37-5,8). We also compared the data obtained at collection and after 30 days of cryopreservation to the results obtained at release. We observed a satisfying recovery for each parameter tested, in line with our expectations, and a reproducibility at every time points of the protocol. These data are shown as percentage of recovery: TNC 88,6% (r. 62-100); TNC viability 69% (42-94); MNC 78% (r. 50-93); CD34+ 71% (r. 43-99). No case of microbial contamination. **Conclusions.** Our protocol allow us to validate the graft, monitoring all the different steps of PBSCs production, from collection until distribution. Furthermore, we have the possibility to keep the critical points of this process under control. Just at collection time we are able to establish the effective performance of cryopreserved PBSCs, particularly referred to the CD34+ cells count. This could be mainly explained by the close correlation existing among MNC collected, cell viability and CD34+ amount.

1888

INFLUENTIAL FACTORS IN THE DAYS OF HOSPITALIZATION FOR THE AUTOLOGOUS STEM CELL TRANSPLANT

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Objective. We analyze the factors of risk that present influence in the increase of the days of hospitalization in patients admitted for an autologous stem cell transplant (autoHSCT). **Methods.** We study 155 patients submitted to autoHSCT in the last 5 years (2007-2011): 103 men (66,5 %) and 52 women (33,5 %) with median age 49 years (SD 12,9). The diagnoses were: monoclonal gammapathy in 43,2 % (67 patiens), lymphoid malignancies in 48,4 % (75 patients), acute leukemia in 7,1 % (11 patients) and other diseases 1,3 % (2 patients). Before to the autoHSCT. 39,5 % (61 patients) had more of two lines of treatment. About of the status of the disease pre-trasplant: complete remission in 75 patients (48,4 %), partial remission in 62 patients (40 %) and disease in progression 18 patients (11,6 %). We realize univariate and multivariate analysis with the following factors: age, sex, diagnosis, lines of treatment before autoHSCT, Sorror index, status pre-trasplant, use of total body irradiation in the conditioning, time of recovery of neutrophil (>0,5 x10^9/L two consecutive days), need of total parenteral nutrition (TPN), infection and the engrafment syndrome. **Results.** With the multivariate analysis, we find the following significant factors: diagnosis (RR=6,75), Sorror index (RR=3,07), status pre-trasplant (RR=0,4), use of TPN (RR=3,13) and the infection (RR=9,81). Other studied factors were not significant. With more of 20 days of hospitalization: the patiens with monoclonal gammapathy, lymphoid malignancies and acute leukemia in a 38,8 %, 78,7 % and 81,8 %, respectively (p<0,002); the patients with Sorror index major or equal 1, 63,5 % (Vs. 58 % Sorror index=0), (p<0,10); complete remission, partial remission and disease in progression with 70,7 %, 48,4 % and 66,7 %, respectively (p=0,06); the TPN more tan 7 days, 77,5 % (Vs. TPN < 7 days: 62,9 %; p<0,10) and the infection during the autoHSCT, 67,5 % Vs. no infection 37,5 % (p<0,05). **Conclusions.** In our series were determinant factors in the increase of the number of days of hospitalization the diagnosis, the Sorror index, the status pre-trasplant, the use of TPN and the infection. We do not find statistically significant relation when we analyze the sex, the age, the previous lines of treatment, the use of corporal total irradiation in the conditioning, the time of recovery of the neutrophils and the engranfment síndrome.

1889

USE OR RITUXIMAB IN ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD) POST ALLOGENEIC HEMATOPOIETIC CELL-TRANSPLANTATION

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Background. Graft-versus-host disease is a well recognized and frequent complication after allogeneic hematopoietic cell transplantation (HCT). It carries significant morbidity and mortality particularly if steroid-refractory. There is evidence to suggest the involvement of B-cells in the pathophysiology of GVHD, particularly in chronic GVHD. The use of Rituximab, a monoclonal anti-CD20 antibody, in the treatment of GVHD, has limited evidence. Observational studies have shown a clinical response rate of up to 70% in patients treated with Rituximab for steroid-refractory chronic GVHD. **Aims.** We reviewed all of our patients treated with Rituximab for either acute or chronic GVHD post allogeneic HSCT between January 2005 and January 2012. **Methods.** Our electronic chemotherapy prescribing system was used to identify all patients who had rituximab for this

indication. The clinical notes were used to extract data on patient demographics, transplant details, presentation and grading of GVHD, the organ(s) affected and timing and outcome of Rituximab use. **Results.** Out of a total 274 allogeneic HCTs (235 mini-allogeneic HCTs) Rituximab was used for treatment of GVHD in 16 patients (6%), all post reduced intensity conditioning (RIC) transplants. For all 16 patients peripheral blood stem cells were the source. Only one patient received a Campath containing conditioning regimen. GVHD-grading is assumed severe though poorly documented. 7 patients (44%) had acute GVHD (skin, gut and a combination of both); 9 (56%) patients had chronic GVHD (8 skin, 2 in combination with liver and gut, and 1 liver). Response was poor in almost all patients: 5 patients died during Rituximab treatment (31%), all in the acute GVHD setting. Only 2 patients (13%) had a documented response: 1 in chronic GVHD (and remains well) and 1 acute GVHD although this patient went on to have two further lines of GVHD treatment. The remaining 9 patients (56%) had no documented response and received further lines of treatment. There was no consistency in agents used after initial ciclosporin and steroids treatment failed. Rituximab was third or fourth line treatment in all cases. Four patients (25%) are currently alive (mean age 36 years). The median overall survival (OS) post-transplant was 23 months. The median time from development of GVHD to commencing Rituximab was 2 months. In the 12 deceased patients (75%) the median time from rituximab treatment to death was 5 months; their mean age was 56 years. None of the patients with gut GVHD survived, either acute or chronic. **Conclusions.** Response to Rituximab was disappointing in both acute and chronic GVHD. In the acute GVHD setting no significant benefit was seen and the majority of patients died during or within a month of Rituximab therapy, reflecting the high mortality associated with acute, severe steroid-refractory GVHD. In the chronic GVHD setting as well there was no significant benefit seen. It has to be acknowledged however that no clear consistency was seen in the prescribing pattern of Rituximab with regard to when (which line) and for which indication to prescribe it; This is in keeping with the current lack of strong evidence or guidelines.

1890

SAFETY AND EFFICACY OF EAM REGIMEN FOLLOWED BY AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR RELAPSED AND REFRACTORY HODGKIN LYMPHOMA ADULTS

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Objectives. The efficacy of high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) for refractory or relapsed Hodgkin's lymphoma (HL) has been reported, but an optimal conditioning regimen has not been determined. Several chemotherapy preparative regimens are used, however, there has not been a randomized clinical trial to support the superiority of one regimen over another. We report treatment results achieved for refractory or relapsed HL in adult patients with a new conditioning regimen. Patients and **Methods.** From March to December 2011, all patients with HL were treated with high-dose Etoposide (800 mg/m²), Aracytine (8g/m²) and Melphalen (140 mg/m²) (EAM) followed by reinfusion of peripheral ASCT. **Results.** A total of 18 patients were retrospectively evaluated. The median age was 27 years (range; 17-47), median number of CD34+ cells was 3, 86 x 10^6/l (2,71 - 6,15). All patients had a full haematopoietic reconstitution. Median time to achieve neutrophils > 500 /µl was 13 days (range; 10-19) and median time to achieve an unsupported platelet count >20000/µl was16 days (range; 14-25). Toxicities included grade 4 hematologic in 18/18 patients, grade 3 mucositis in 4, grade 3 infectious in 2. No case of transplant-related mortality occurred. After a median follow up of 8 months, all patients are alive and all are in continuous CR. In conclusion, the EAM regimen would be an effective and tolerable conditioning regimen with acceptable engraftment and toxicity for ASCT for refractory or relapsed HL. Although these outcomes are encouraging, longer follow-up is required and comparison with other traditional ASCT regimens used for patients with refractory or relapsed HL is warranted.

1891

FAVORABLE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA USING FLUDARABINE, CYCLOPHOSPHAMIDE, ATG WITH OR WITHOUT LOW-DOSE TBI

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Background. Because of the toxicity and high rate of graft rejection, the standard conditioning regimen for severe aplastic anemia (SAA) has yet to be established, and a variety of reduced-intensity conditioning regimens using fludarabine have been reported. **Aims.** We have retrospectively evaluated the safety and efficacy of allogeneic HSCT for SAA using fludarabine, cyclophosphamide, and ATG with or without low-dose TBI. **Patients and Methods.** Six patients (median age 30 (range: 22-61)) with SAA who underwent allogeneic bone marrow transplantation from an HLA-identical sibling (n=3) or an HLA-matched unrelated donor (n=3) were evaluated. Conditioning included fludarabine (120 mg/m^2), cyclophosphamide (100 mg/kg), and ATG (Thymoglobulin; 3. 75 mg/kg). In addition, 2 Gy of TBI (with ovarian shielding for young female patients) was delivered for heavily transfused patients or HSCT from an unrelated donor. For the prophylaxis of graft-versus-host disease (GVHD), cyclosporine A or tacrolimus with short-term methotrexate was given. **Results.** The transplant procedure was generally well-tolerated, and there were no life-threatening complications. All patients achieved engraftment and became transfusion independent. Full donor chimerism was confirmed at day 28 after transplantation. Only one patient developed acute GVHD (grade II) and none developed chronic GVHD. With a median follow-up period of 6. 8 months (range: 4. 4-14. 3 months), all patients are alive and in good performance status. The recovery of menstruation was observed in all 3 evaluable young female patients at 3, 7, and 8 months after transplantation, respectively. **Conclusions.** These results suggest that fludarabine, cyclophosphamide, and ATG with or without low-dose TBI could be a promising conditioning of allogeneic HSCT for SAA both from sibling and unrelated donor. In addition, this conditioning with ovarian shielding in TBI is considered to have a minimal adverse effect on ovarian function in female patients.

1892

PNEUMONIA IN AUTOLOGOUS STEM CELL TRANPLANTATION RECIPIENTS: DOES IT REPRESENT NEGATIVE PROGNOSTIC FACTOR FOR OUTCOME?

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Background. Autologous stem cell transplantation (ASCT) has become a curative therapy for the majority of Hematologic malignancies. Infections remain the most significanti causes of morbidity and mortality. **Aims.** the aim of our study was to describe incidence, etiology, mortality and risk factors of infections and mainly of pneumonia complications in our series of patients. **Methods.** in our institution 173 patients underwent ASCT from March 2005 to December 2011. 108 were male and 65 were female. Median age was 54 (range 16-74). Underlying disease, conditioning regimen, status of disease at the time of transplantation, number of previous therapies are described in Table 1.

Table 1. Patients characteristics.

N patients 173		
Sex	Male 108 (62%)	Female 65 (38%)
Age	Median	54
	Range	16-76
Underlying disease	NHL + HD	96 (55.5%)
	MM	68 (39.3%)
	AML	3 (1.7%)
	OTHER	6 (3.5%)
Disease status at transplant	Responsive	146 (85%)
	Unresponsive	27 (15%)
Conditioning Regimen	BEAM or BEAM like	69 (40%)
	MELPHALAN	71 (41%)
	Z BEAM	19 (11%)
	OTHER	14 (8%)
Number of previous therapy	Median	2
	Range	1-4
Febrile Neutropenia	106 (62%)	
Days of Neutropenia	Median	10
	Range	7-29
Type of Fever	FUO	44 (41.5%)
	Micro biol. documented	43 (40.5%)
	Pneumonia	19 (18%)

Median duration of neutropenia was 10 days (range: 7-29). Infectious complications occurred in 106 patients: 44 FUO, 43 micro biologically documented fever, 19 pulmonary infections. 7 of these were micro biologically documented: 2 Pseudomanas Aeruginosa, 2 Escherichia Coli, 2 Staphilococcus Hominis, 1 Klebsiella Pneumoniae. No pulmonary Aspergillosis was documented. **Results.** We analyzed the impact of some clinical parameter on the occurrence of infection and in particular of pneumonia. There are no statistically differences concerning the type of conditioning regimen, the underlying disease, mucositis, age > 55 years, gender, sex, number of prior lines of chemotherapy and the duration of neutrope-

nia (more or less than 10 days)Moreover we evaluated the differences in terms of transplant related mortality (TRM). TRM in our series is 8% at 30 days and 12. 7% at 100 days. On the other and the TRM of patients who developed any infections is statistically significant higher both at 30 days (13% versus 0% p 0. 005) than at 100 days (19% versus 2% p 0. 002). In this setting of patients the difference of TRM between pneumonia and other infectious complications was analyzed. TRM at 30 days was respectively 15. 6% versus 12. 6% (p 0. 7); TRM at 100 days was 26. . 3 versus 17. 2 (p 0. 3). **Summary.** infections are the major causes of morbidity and mortality in patients with different hematological diseases after ASCT. The incidence of pneumonia in our series seems higer than in other cohort of patients described in litterature (18% versus 8%) but it doesn't seem to correlate with worse TRM and outcome . Now we are planning a multicenter study to better detail the incidence of pneumonia and correlated risk factors after ASCT.

1893

HALLUCINATION AND INSOMNIA AS A SIDE EFFECT OF PLERIXAFOR IN A 3-YEAR-OLD GIRL WITH RELAPSED WILMS TUMOR IN THE SETTING OF NEPHROBLASTOMATOSIS

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Background. After several randomized studies, plerixafor was approved in adult patients in 2009 by the European Medicines Agency for use in combination with filgrastim to enhance mobilization of hematopoietic stem cells to peripheral blood. Plerixafor reversibly inhibits stromal cell-derived factor-1-a/CXCR4 binding, as a result of which CD34+ cells are mobilized into circulation. **Aims.** Although the treatment is well tolerated in adult population, some adverse effects such as diarrhea, nausea, vomiting, fatigue, joint pain, headache, dizziness, anxiety, sleeplessness, injection-site redness, and irritation have been reported. Experience in children, however, is extremely limited. We report our experience in 3-years-old girl with relapsed Wilms tumor in the use of this drug. **Case Report.** A 3 year-old girl admitted to hospital with abdominal pain. On physical examination, a left abdominal mass was discovered. Abdominal ultrasound showed three mass at the left kidney. She was operated and the mass was completely resected. The pathological examination showed Wilms tumor in the setting of nephroblastomatosis According to radiological and pathological findings she was diagnosed as stage IV Wilms tumor with unfavorable histology. She underwent a radiation and chemotherapy protocol for Wilms tumor recommended by Turkish Pediatric Oncology Group. At 7th month of the planned chemotherapy the patient showed multiple metastases at liver, local radiation to liver relapse was performed and 2 courses Ifosfamide, carboplatin and etoposide (ICE) was given. After the 2nd course of ICE it was failed to mobilize the hematopoietic stem with filgrastim; so after obtaining parent's informed consent form, plerixafor was administered subcutaneously (240 μg/kg) prior to apheresis. The mobilization was performed successfully but the patient showed hallucination and insomnia after 12 hours of the administration of plerixafor. The blood electrolytes, EEG, thyroid function test and brain MRI found to be normal. The patient underwent hydroxyzine treatment for two days and the symptoms disappeared within 48 hours. **Results.** According to our knowledge, we report the first pediatric experience of hallucination and insomnia as a side effect of plerixafor in the English medical literature. **Summary and Conclusions.** This case illustrates that the plerixafor is an effective agent for mobilization of hematopoietic stem cells in children; however clinicians must be vigilant about the possible side effect of hallucination and insomnia. Further studies are necessary to evaluate this side effect in childhood.

1894

GUILLAIN-BARRE SYNDROME AFTER STEM CELL TRANSPLANTATION: SINGLE CENTER EXPERIENCE

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Background. Guillain-Barre syndrome (GBS) is a uncommon and unusual complication in the setting of solid organ and hematopoietic stem cell transplantation (HSCT). We intend to report three cases of GBS following autologous and allogeneic HSCT. **Method.** We retrospectively reviewed our transplantion records. Three patients out of 517 transplants (271 allogeneic HSCTs, 245 autologous HSCTs) carried out between 1993-2011 developed GBS posttransplant. Demographic features, clinical courses, predisposing conditions and management strategies are summarized (Table 1). **Results.** Case 1: A 57-year-old male patient diagnosed with Hodgkin's Disease underwent autologous HSCT. He suffered from frequent upper tract respiratory infections posttransplant. Five months after HSCT, a diagnosis of GBS was made on development of sensory-motor lower extremity-limited demyelinating polyneuropathy. He was treated with intravenous immunoglobulin (IVIG) with significant improvementCase 2: A 20-year-old male with a diagnosis of acute myeloid leukemia underwent HSCT from full-match sibling donor. He presented with acute GVHD involving skin, oral mucosa and liver after 7 months posttransplant. He was treated with high dose methylprednisole and cyclosporine. Pneumonia and cytomegalovirus reactivation ensued and treated accordingly. Infectious complications prolonged hospital stay. Two months after his admisson, parasthesia accompanied by ascending motor weakness of four extremities and peripheral fascial paralysis developed, leading to inability to walk without support at the end of one week. He was treated with IVIG 400 mg/m2 for 5 days. We proceeded to plasmapheresis as a result of unresponsiveness to IVIG after 2 weeks. The patient responded dramatically with full motor recovery. With regard to etiological factors, GBS was preceded with lower respiratory and urinary tract infections. Serological markers were negative all but for anti-CMV IgM positivity whereas CMV-DNA was negative. Case 3: A 37 year-old female, diagnosed with Ph (+) acute lymphoblastic leukemia, recieved allogeneic HSCT from her full-match sist er. Acute GVHD developed in early period with complete resolution following therapy. She was diagnosed with GBS involving lower extremities, leading inability to walk and take care of herself. IVIG 400 mg/m2 for 5 days was commenced. The patient responden gradully and began walking without assistance one week later. No antecedent infection was detected. **Discussion.** GBS is a heterogenous disorder mediated by a multitude of pathogenic mechanism and exceptionally rare following HSCT. Different underlying mechanisms following HSCT may contribute to development of GBS. A higher rate of infections and GVHD may predispose to GBS posttransplant. IVIG and plasmapheresis remain the treatment of choice for patients recieving HSCT. Therapy should be implemented effective immediately.

Table 1. Demographic and clinical features.

Patient	Age/Sex	Diagnosis	Type of HSCT	Time interaval	Conditioning Regimen	GVHD	Predisposing condition	Management
1	57 male	Hodgkin's Disease	Autologous PBSCT	5 month	BEAM	Nonapplicable	Acute sinusitis	IVIG
2	20 male	AML	Allo-HSCT BM	7 months	BU/CY	Skin oral mucosa, liver aGVHD	Respiratory, urinary infections, CMV reactivation ?	IVIG + plasmapheresis
3	37 female	Ph+ ALL	Allo-HSCT BM	12 months	BU/CY	Skin, acute GVHD	None	IVIG

1895

EFFECT OF GRANULOCYTE COLONY-STIMULATING FACTOR MOBILIZATION ON THE EXPRESSION PATTERNS AND CLONALITY OF TRAV AND TRBV REPERTOIRE

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Background. The immune modulatory effect of granulocyte colony-stimulating factor (G-CSF) on T cells resulted in an unexpected low incidence of graft-versus-host disease (GVHD) in allogeneic peripheral blood stem cell transplantation (allo-PBSCT). Recently, αβ+T cells are identified as the primary effector cells for GVHD. However, whether G-CSF could influence the repertoire of αβ+ T cells (TRAV and TRBV repertoire) remains unclear. **Aims.** To investigate the effect of G-CSF mobilization on the T cell receptors (TCR) of αβ+ T cells (TRAV and TRBV repertoire), as well as the association between the changes of TCR repertoire and

GVHD in patients undergoing G-CSF mobilized allo-PBSCT. **Methods.** The complementarity-determining region 3 (CDR3) sizes of 29 TRAV and 24 TRBV subfamily genes were analyzed in peripheral blood mononuclear cells (PBMCs) from 20 donors before and after G-CSF mobilization, using RT-PCR and genescan technique. **Results.** The numbers of detectable TRAV and TRBV subfamilies, as well as the expression frequencies of most TRAV and TRBV subfamilies decreased at different levels after G-CSF mobilization. The expression frequency of TRAV27 after mobilization was significantly lower than that before mobilization (P=0. 031). Most TRAV and TRBV subfamilies revealed polyclonality from pre-G-CSF and G-CSF-mobilized samples. Oligoclonality was detected in TRAV and TRBV subfamilies in 2 donors before mobilization and 6 donors after mobilization, predominantly distributed in TRBV16. Significant positive association was observed between the variable clonality of TRBV22 gene repertoire after mobilization and low incidence of GVHD in recipients (P=0. 042, OR=12. 500). **Conclusions.** G-CSF mobilization has an effect on the expression patterns and clonality of *TRAV* and *TRBV* repertoire. This alteration might play a role in mediating GVHD in G-CSF mobilized allo-PBSCT.

1896

T CELL RECEPTOR V? REPERTOIRE WAS DIFFERENTLY USED BETWEEN GRAFT-VERSUS-HOST DISEASE AND GRAFT-VERSUS-LEUKEMIA IN ACUTE LYMPHOCYTIC LEUKEMIA WITH TCF3-PBX1

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Background and Aims. Many clinical reports indicate that graft-versus-host disease (GVHD) and graft-versus-leukemia (GVL) are difficult to be separated, and sometimes GVHD becomes serious when a transplanted patient relapses and immunosuppressant is reduced to induce GVL effect. We recently experienced one acute lymphocytic leukemia (ALL) case with TCF3-PBX1 who relapsed after allogeneic hematopoietic stem cell transplantation (HSCT). This patient achieved complete remission (CR) with a discontinuation of immunosuppressant. Fortunately, the patient's GVHD was controllable, and the patient is still alive and well. We analyzed blood T cell receptors (TCRs) obtained from this patient to understand better GVHD and GVL. Case: A 40 year-old Japanese man was suffered from ALL with TCF3-PBX1 in November, 2009. He was treated with chemotherapy, and achieved CR; however, before allogeneic HSCT his ALL relapsed. He was transplanted with unrelated bone marrow donor from JMDP in June, 2010, and achieved CR. On day 200 his ALL relapsed, and cyclosporine-A and prednisolone were stopped. Soon after a discontinuation of immunosuppressant he achieved CR. GVHD worsened to grade III in liver, and immunosuppressant started again. His GVHD was well-controlled, and his ALL maintains CR until now. Materials and **Methods.** The institutional ethical committee approved this study, and he was given informed consent. His leukemic blasts, blood after achieving CR, and liver biopsy specimen when his GVHD worsened, were collected. Blood lymphocytes were cultured with the irradiated his ALL blasts (GVL model) or liver cells (GVHD model) in RPMI1640 medium with 10% FCS and 100 ng/mL of recombinant human interleukin-2 for 14 days. Then RNA was extracted, and TCR Vα and Vβ usages were analyzed with RT-PCR using the specific primers for subfamilies and the constant region of α or β. **Results.** In GVHD model and GVL one, TCR Vα and Vβ were oligoclonally used. Several subfamilies were demonstrated in both models. Vα10, 11, and 12 were preferentially used in GVHD model but not in GVL. Vβ 2, 4, and 5. 1 were observed in GVHD model but not in GVL. And, Vβ 6, 7, 10, 12, and 14 were demonstrated in GVL model but not in GVHD. Discussion: In both GVHD and GVL model TCR Vα and Vβ were preferentially used, and some difference was observed. Importantly, some Vβs were used in GVL model but not in GVHD. We previously reported that vascular endothelial growth factor C, its receptor type-3, and lymphduct neogenesis-related genes were expressed in ALL with TCF3-PBX1. These proteins may contribute to the preferential TCR Vβ usage in GVL model. We now isolate single T cell clone that induces GVL effect but not GVHD, and determine the leukemia-specific antigen for GVL effect.

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ASSOCIATION OF MEAN PLATELET VOLUME WITH NEONATAL INNATE FACTORS AND THE NUMBER OF TOTAL NUCLEATED CELLS AND CD34+ CELLS IN CORD BLOOD

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Background. Cord blood (CB) is an attractive stem cell source compared to bone marrow (BM) or peripheral blood (PB) in hematopoietic stem cell transplantation. Mean platelet volume (MPV), which is a measure of platelet size, is a potential marker of platelet activation. It has been recently reported that MPV of CB correlates with the hematopoietic parameters, such as the numbers of TNC and CD34+ cells. Therefore, MPV are considered as one of the hematopoietic parameters of CB. **Aims.** We investigated whether the MPV of CB is associated with the hematopoietic parameters of CB donated by Korean neonates and maternal and neonatal factors. **Methods.** A total of 10,577 units were enrolled among CB donated to the Seoul Metropolitan Public Cord Blood Bank (Allcord), during the period from October 2006 through August 2009. The enrolled CB units were donated from Korean. We measured the associations between the MPV of CB and CB volume, the numbers of total nucleated cells (TNCs), CD34+ cells, and CD34+ cells/TNCs in CB; and the influence of maternal age and neonatal factors, such as gender of neonates, gestational age, and birth weight on the MPV of CB. We also analyzed these associations and influences according to the ABO blood groups of the neonates. **Results.** MPV of CB significantly correlated with the CB volume (r = 0. 121, $P < 0.001$), and with hematopoietic parameters, such as the numbers of TNCs (r = 0. 110, $P < 0.001$), CD34+ cells (r = 0. 174, $P < 0.001$), and CD34+ cells/TNCs (r = 0. 157, $P < 0.001$). Although the MPV showed no association with maternal age (r = 0. 003, p = 0. 739) and gender of the neonates (male, 9. 479 ± 0. 6092 fL; female, 9. 479 ± 0. 6149 fL), it showed a significant negative correlation with the gestation age (r = -0. 102, $P < 0.001$) and a significant positive correlation with the birth weight (r = 0. 023, $P = 0.020$). In analysis according to the ABO blood groups of neonates, CB blood type O showed higher MPVs ($P = 0.002$) than other blood types of CB, especially comparing with the neonates of blood type B. Also, CB blood type O showed higher numbers of TNCs ($P = 0.013$), CD34+ cells ($P < 0.001$), and CD34+ cells/TNCs ($P < 0.001$) than other blood types of CB. The neonates of blood type O showed significantly high number of TNC, especially comparing with the neonates of blood type A. In comparing the number of CD34+ cells of CB unit, the neonates of blood type O showed significantly higher number than the other blood types. Also, the neonates of blood type O showed significantly higher CD34+ cells/TNC than the other blood types. **Conclusions.** Considering these associations of MPV with hematopoietic parameters and the influences of neonatal factors on the MPV, MPV can be considered as a hematopoietic parameter of CB.

1898

BONE MARROW SEEMS TO BE THE BEST SOURCE OF MESENCHYMAL STEM CELLS TO REPAIR INJURED LIVER

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Background. Several genetic hepatic metabolic diseases alter physical and neurological development as well as life expectancy of affected children. The only potential curative option to date for these patients is a liver transplant. Given the shortage of organs, development of cellular sources other than human liver is urgent. **Aims.** The main objective of this project is to demonstrate the feasibility of treating liver metabolic diseases by MSC transplantation. **Methods.** Human MSC from umbilical cord (UC-MSC), bone marrow (BM-MSC) or liver (L-MSC) were transplanted into NSG mice after CCl_4-induced liver injury. In order to support MSC homing towards injured liver, we induced expression of the CXCR4 receptor on their surface. NSG mice received 3 CCl_4 3% IP injections per week during 4 weeks. 48h after the last injection, mice received 500,000 MSC by intravenous tail injection. We injected UC-MSC, BM-MSC or L-MSC (CXCR4- or CXCR4+). We examined MSC homing by real-time PCR and MSC function by quantitative image analysis of sirius red staining and blood enzyme analysis. **Results and Conclusions.** Data confirm that CCl_4 treatment induced hepatic fibrosis. PCR showed that human MSC, after injection in mice, were found partly in their liver. In addition, BM-MSC seemed to be the most promising cells. Indeed, they stabilized the rates of plasma albumin and alanine amino-transferase of mice. Moreover, these cells decreased hepatic fibrosis. CXCR4 expression did not improve the homing nor function of MSC. BM-MSC CXCR4+ or UC-MSC or L-MSC (expressing CXCR4 or not) seemed less effective. These results need to be confirmed in a larger number of animals.

1899

MIGRATION AND DIFFERENTIATION OF TRANSPLANTED BONE MARROW-DERIVED CELLS INTO PERIODONTAL TISSUES PROMOTED BY MECHANICAL STRESS

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Aims. Bone marrow-derived cells have abilities of cell migration and differentiation into teeth and related tissues/organs, especially into periodontal ligament

fibroblast cells [1,2]. In this examination using a bone marrow transplantation model, we examined the effect of orthodontic mechanical stress to the transplanted bone marrow-derived cell migration into periodontal tissues. **Methods.** Bone marrow derived cells from green fluorescence protein (GFP) transgenic mice were transplanted into 8 week-old female C57BL/6 immunocompromised recipient mice (n=10), which had undergone 10 Gy of lethal whole-body irradiation. After successful transplantation (about one month period), 5 mice received orthodontic mechanical stress using the Waldo method 5 times in 5 weeks; and 5 mice were compared as control without receiving orthodontic mechanical stress. After that, the regional tissues were removed and fixed in formalin fixative. Paraffin-embedded sections were immunohistochemically analyzed using a Dako Envision + Kit-K4006 (Dako,Glostrup,Denmark) and a primary anti-GFP-polyclonal rabbit antibody (#598; 1/500; MBL,Nagoya,Japan). For semiquantitative evaluation of immunohistochemical staining, the following procedures were performed. First immunohistochemical images with same magnification from the periodontal tissues were prepared and pixel density was counted for each image. Then typical immunohistochemically positive staining part was defined as positive area. The pixel number of positive area in the periodontal tissue was compared with the previously calculated total pixel number of the periodontal tissue and the ratio was obtained. **Results and Discussion.** We examined the transplanted bone marrow-derived cell migration into periodontal tissues. The immunohistochemistry revealed that GFP-positive cells were detected in the periodontal tissues, both in the experimental and control specimens. The GFP-positive cells histopathologically differentiated into some cell types. The fluorescence IHC and TRAP staining techniques demonstrated these cells were detected as osteoclasts and macrophages. Furthermore, GFP-positive cells gathered adjacent blood vessels. The data suggest that GFP-positive bone marrow-derived cell migrate into periodontal tissues and differentiate periodontal tissue component-cells. In the experimental group, there were numerous GFP-positive cells appearing in the experimental periodontal tissues which received intermittent stimulation of orthodontic mechanical stress, but there were few GFP-positive cells in the control specimens. As a results of the examination group specimens and control group, the ratio of pixel number in the examination group showed 5. 77 ± 3. 24 % (mean ± SD); and that in the control group, 0. 71±0. 45 % (mean ± SD). The examination group was significantly greater than that of control group (Mann-Whitney U test: p<0. 001). Thus, these data indicated that orthodontic mechanical stress acts as a possible promoting factor of transplanted bone marrow-derived cell migration into periodontal tissues, and of differentiation to fibroblasts. **Conclusions.** These results suggest that orthodontic mechanical stress induces transplanted bone marrow-derived cell migration and differentiation into periodontal tissue.

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1900

OPTIMIZATION OF CRYOPRESERVATION CONDITIONS FOR MESENCHYMAL STEM CELLS USED FOR BONE REPAIR

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Background. The utilization of tissue engineering in the repair of bone defects has shown great promise recently. In combination with appropriate biomaterials and growth factors, bone marrow-derived mesenchymal stem cells (MSC) have been proven to significantly enhance bone repair in large animal fracture models. MSC banking is feasible but the optimal technique of cryopreservation must be developed. **Aims.** First, we tested different cryoprotectants (CPA) (DMSO and/or trehalose and/or sucrose) and different concentrations. Then, we studied speed of freezing process as well as technique of elimination of CPA after thawing process. **Methods.** $2x10^6$ cells were transferred to a cryovial containing each CPA solution. Cryovials were immediately frozen at -80°C during 24h then transferred into a liquid nitrogen cylinder at -196°C. After 1 week, the vials were removed from liquid nitrogen, placed in a 37°C water bath. Then, cellular suspension was washed twice with cold culture medium. Viability was analyzed with a Trypan blue dye exclusion assay. Cell proliferation of cryopreserved MSC was determined after 7 days of culture. **Results.** No significant differences in viability percentage were detected among cryopreservation solutions with 5% and 10% DMSO independent of addition of trehalose or sucrose. When cells were cryopreserved with 2,5% DMSO, fewer than 30% of MSC were viable. Proliferation didn't change significantly after thawing process in 15 media tested. However, proliferation tends to be more important when MSC are frozen in 5% DMSO + trehalose. When MSC were freezed with a freezing container (-1°C/min) and when DMSO were washed drop by drop, viability percentage reached more than 90%. **Conclusions.** In conclusion, it would be possible to replace standard CPA (10% DMSO) by a solution with 5% DMSO + 60mM trehalose. It is preferable to freeze the cells in a progressive way and to wash slowly the cells.

1901

THROMBIN GENERATION AND CONVENTIONAL COAGULATION MARKERS IN PATIENTS WITH LOW PT AND/OR APTT

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Background. The endogenous thrombin potential (ETP) represents the balance between pro- and anti-coagulable processes in plasma and can be used to identify hypo- and hyper-coagulabiltiy. The ETP assay offers a new measure of global coagulability. The ETP Assay is a functional assay for quantification of thrombin generation capacity. The evaluation of the reaction kinetic of the thrombin generation allows potential value for clinical diagnosis, drug monitoring and epidemiology. Recently a test has become available to routinely measure the endogenous thrombin generation potential (ETP) by Dade Behring (Germany). Results are automatically calculated by the BCS® System with the ETP analysis software. Some investigators believe that patients with low values of conventional coagulation tests (PT/INR, aPTT) have an increased incidence of thrombosis. Extensive coagulation studies have not yet been deriving a stable model of thrombosis in those patients. As a preliminary step to larger clinical studies we investigated the effect on ETP values of patients presenting low values on Conventional coagulation tests (PT/INR, aPTT). **Aims.** The comparison of ETP values and conventional coagulation markers between controls and patients with low PT and/or aPTT. **Methods.** 120 samples, of consecutive patients with low values of conventional coagulation tests PT/INR and/ or aPTT, and 30 samples of controls were investigated for PT/INR, APTT, and ETP parameters. We considered low values for INR less than 1 and aPTT lower than 26 seconds. We used the chromogenic method on the fully automated Behring Coagulation System (BCS) for the measurement of thrombin generation parameters. **Results.**The patients were 45 males and 75 females . There were no significant differences between them according either conventional coagulation tests or ETP parameters. We had 32 patients with low PT/INR and aPTT, 104 patients with both low PT/INR, and 43 patients with aPTT values less than 26 seconds and 120 patients with low PT/INR and / or aPTT. Although all the results were significant, except Cmax parameter, the most significant differences were observed in low PT/INR group. (Table 1). **Summary and Conclusions.** The automated ETP test can play an important role in the evaluation of haemostatic function in patients with low values in conventional coagulation tests. A potential clinical implication of these findings is that the laboratory investigation of the coagulation function, presently performed with the PT and APTT, is inadequate to assess the true risk of thrombosis. Perhaps the measurement of thrombin generation might be more suitable to evaluate the thrombotic risk. Although plausible, this hypothesis needs to be sustained clinically by a prospective study.

Table 1.

	30 CONTROLS		120 PATIENTS PT/INR and/ or aPTT		
	mean	sd	mean	sd	p
tlagsec	19.3	2.6	29	16.5	0.002
tmax sec	54.3	4.2	75.5	26.9	0.0001
CmaxmA/min	123.5	6	115.1	43.7	0.293
ETPmA	394.7	29.5	434.4	73.1	0.0001
PT	10.8	0.6	10.4	0.6	0.01
INR	0.9	0.06	0.85	0.05	0.0001
APTT	30.7	2.7	28	4	0.001

1902

MANIFESTATION AGE OF THROMBOLISM IN THROMOPHILIA

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Background. Thrombophilia is diagnosed in 45-50 % of patients suffering from thromboembolic events. More than 8% of thrombosis cases present combined hereditary thrombophilic defects. For most of them, life-long treatment with anticoagulants is recommended. Severity of thrombophilia can be calculated from the manifestation age of thromboembolism. Different thrombophilic states were examined to find out whether combined defects really are more dangerous than single defects really and do require life long anticoaguagulation. **Aims.** To report our experience of treating 655 patients (150 male, 505 female), with an average age at diagnostic of 41 ± 12years. **Methods.** In all patients, we determined fibrinogen, antithrombin III, PT, APTT, protein C, protein S, antithiphospholipid antibodies, factor V Leyden, homocysteine, prothrombin G 20210 A, and MT HRF (667 T)-polymorphisms were checked. **Results.** Thrombophilic defects were detected in 241 patients;MTHFR polymorphism and hyperhomocysteinemia were found in 67 of them. 83 patients presented factor V mutation, in 24 protein S was deficient, in 12 protein C was deficient, in 13 patients prothrombin polymorphism was present and in 42 anthiphospholipid syndrome was detected. Six patients had a combination of three and 19 of two such defects. Manifestation age of thromboembolism in patients without coagulation defects was of 41 ± 12 years. In patients with coagulation defects, manifestation age was not significantly earlier, except for patients with anthithrombin deficiency (16 ± 2 years). Patients with combined defects presented a manifestation age of 32 ± 12 years (3 defects) and 33 ± 11 years (2 defects). **Conclusions.** Based on the manifestation age of thromboembolism, combined thrombophilic defects do not seem to be more severe than single defects. Therefore, life-long oral anticoagulants are not necessary in every patient with combined thrombophilic defects.

1903

CONGENITAL CAVAL AND PELVIC VEIN ABNORMALITIES AS A RISK FACTOR FOR RECURRENT DVT IN YOUNG PATIENTS

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Background. During the embryogenesis, the inferior vena cava (IVC) develops from 3 pairs of embryonic veins. Disruption of this process may result in condition known as IVC agenesis. Idiopathic deep venous thrombosis (DVT) presents a clinical challenge regarding duration of anticoagulation therapy, when no apparent risk factors are identified. When a young patient presents with extensive DVT, congenital anomalies of the IVC preventing efficient venous blood flow from lower extremities may be the reason. **Aims.** We report 5 patients presenting with juvenile idiopathic DVT without apparent family history of venous thrombosis. **Methods.** Retrospective chart review. **Results.** See attached Table 1 for the clinical details that established the diagnosis of congenital IVC anomaly.

Table 1.

Patient (sex)	Age at presentation (years)	Presentation	Provoking factors	Anatomy
DB (M)	24	Bilateral DVT of common iliac veins	None	Absence of the subrenal segment of the IVC
	28	Bilateral DVT of common iliac, internal and external iliac, and common femoral veins	Prolonged travel	
DT (F)	19	Bilateral DVT of femoral veins	OCP	Azygos continuation of the IVC
	21	Left lower extremity DVT	None	
BH (M)	Newborn	Renal vein thrombosis	None	Azygos continuation of the IVC
	13, 14	Left iliofemoral thrombosis, with recurrence	None	
	15	Right lower extremity DVT	None	
	23	DVT of right popliteal and distal femoral veins	None	
SB (F)	13	Left common iliac vein thrombosis, including femoral, saphenous veins	None	Azygos continuation of the IVC
	16	Bilateral DVT of common iliac veins, including chronic thrombus in IVC	None	
AS (F)	29	Bilateral DVT of common iliac veins	Prolonged travel	Hypoplasia both common iliac veins

Conclusions. Young patients presenting with idiopathic DVT require extensive workup including hereditary thrombophilia, autoimmune and neoplastic disorders. Inferior vena cava agenesis is another risk factor that should be considered, especially in patients without family history of venous thrombosis or distinct thrombophilia. Imaging studies, such as CTA venography, should be included in the workup. In majority of cases long-term anti-thrombotic therapy is warranted.

1904

LUPUS ANTICOAGULANTS IN KOREAN PATIENTS WITH ACUTE ISCHEMIC STROKE

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Background. Antiphospholipid antibodies (APAs), including Lupus Anticoagulant (LA), anticardiolipin antibodies and anti beta-2 glycoprotein I (ab2-GPI), are capable of causing endothelial damage which subsequently can leads to thrombosis. **Aims.** Recently, it has been reported that pediatric cerebellar stroke associated with elevated ab2-GPI. Because cardiovascular involvements are common feature of antiphospholipid syndrome, we studied prevalence rates of LA especially in patients with acute ischemic stroke. **Methods.** Dilute Russell's viper venom time test was done to screen the presence of LA in STA-R (Diagnostica Stago, Inc.). Other autoantibody tests were done using ELISA methods. This study involved 358 patients, 224 males and 134 females (mean age 48. 26 years), with acute ischemic stroke who visited our hospital from August 2008 to July 2011. **Results.** Male to female ratio was 1:1, and the median age was 52. 5 years. In total patients, 8 individuals showed the increased LA result (range 1. 28-67. 5) and 50% of patients (4/8) presented significantly increased LA results (Table 1). Of these patients, one female had rheumatism (multiple sites, unspecified) as an underlying disease and showed all positive results in the 3 kinds of APAs. In these 8 patients with positive LA results, 4 patients had decreased protein S activity levels, and decreased protein C activity levels were found in 2 patients. **Conclusions.** A prospective study should be followed to investigate pathophysiology of LA and other APAs such as ab2-GPI in ischemic stroke. Moreover, further studies are needed to confirm the importance of transient and persistent APAs and their prognostic implications.

Table 1. Characteristics of patients with the positive LA result in 358 patients with acute ischemic stroke.

Case number	Age (y)	Gender	LA**	aCL (U/mL)	ab2-GPI (U/mL)	APA (U/mL)	Anti-dsDNA (U/mL)
1	28	F	+(67.5)	+IgM (40.0)	NA	+IgG (12.0), +IgM (46.0)	-
2	34	F	+(59.4)	-	-	-	-
3	60	M	+(53.3)	-	NA	-	-
4	61	F	+(53.3)	-	NA	-	-
5*	52	F	+(1.87)	+IgG(>100)	+IgG(185)	+IgG(>100)	-
6	48	M	+(1.85)	-	-	-	-
7	57	M	+(1.35)	-	NA	-	-
8	53	M	+(1.28)	+IgG(26.0)	NA	-	-

*This patient had rheumatism (multiple sites, unspecified) as a comorbidity.
** negative cut-offratio <1.2
Abbreviations; LA, Lupus Anticoagulant; aCL, anticardiolipin antibodies; ab2-GPI, anti beta-2 glycoprotein I; APA, antiphospholipid antibody; NA, not available

1905

THROMBOLYSIS WITH RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR IN SEVEN CHILDREN

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Background. The information about the thromboembolic events, the optimal treatment choice, the dose and duration of antithrombotic therapy in children are limited. More clinical data are required. Recombinant tissue plasminogen activator (r-tPA) is increasingly used in pediatric thrombosis. **Results.** We retrospectively analyzed the clinical course of 7 children (9. 3±2. 1 years; 34 day to 16 year) with arterial thrombosis (n:1) and intracardiac thrombosis (n:6). The children were treated with r-tPA. The dose ranged between 0. 2-0. 4mg/kg/h infused for 3 to 4 hours. This dose was repeated between 2 to 7 times till the thrombolysis was achieved. Treatment side effects were closely monitored.

Complete clote lysis was achieved in all cases. None of them had severe bleeding except mild recurrent epistaxis occurring in 2 cases. **Conclusions.** In conclusion, r-tPA is an effective and safe therapy under close haemostatic control in children.

1906

THROMBIN GENERATION PARAMETERS IN VARIOUS HYPERCOAGULABLE STATES

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Background. The endogenous thrombin potential (ETP) represents the balance between pro- and anti-coagulable processes in plasma and can be used to identify hypo- and hyper-coagulabiltiy. The ETP assay offers a new measure of global coagulability. Recently a test has become available to routinely measure the endogenous thrombin generation potential (ETP) by Dade Behring. Acute myocardial infraction (AMI) is a hypercoagulable state. Thrombosis correlates significantly with MM. The role played by coagulation system in multiple myeloma (MM) thrombosis is unclear. People with myeloproliferative disorders (MPD) have an increased incidence of both arterial and venous thrombosis. Some investigators believe that patients with low values of conventional coagulation tests (PT/INR, aPTT) have an increased incidence of thrombosis. Extensive coagulation studies have not yet been derived a stable model of thrombosis in those patients. As a preliminary step to larger clinical studies we investigated the effect on ETP values in those patients. **Aims.** Comparison of ETP values inbetween controls, AMI, MM, MPD and patients with low PT and/or aPTT. **Methods.** 31 patients with AMI were included. Blood was sampled on admission. 18 consecutive samples of MM patients. 24 patients with MPD [10 essential thrombocythaemia (ET), 2 atypical MPDs, 9 polycythaemia vera (PV), 2 chronic myeloid leukaemia (CML) and 1 mastocytosis] were recruited . 120 samples, of consecutive patients with low values of conventional coagulation tests PT/INR and/ or aPTT, and 30 samples of controls were investigated for ETP parameters. We considered low values for INR less than 1 and aPTT lower than 26 seconds. We used the chromogenic method on Behring Coagulation System for the measurement of thrombin generation parameters. **Results.**The results are statistically significant (p< 0. 05) with the exception of ETP in MM and Cmax in low PT/APTT group. MPD patients with ET compared with patients with PV had decreased tlag (24 vs 34. 8), tmax (67. 7 vs 84. 1) but increased Cmax (113. 3 vs 80) and ETP (409. 1 vs 312. 2). ET patients had increased ETP compared to control group (409. 1 vs 395). (Table 1). **Summary and Conclusions.** Although AMI is a hypercoagulable state, AMI ETP is comparable to the values of ETP in controls. ETP appears to take a rather width range of values in AMI. In MM group although ETP had not statistical significance the other parameters were significant. Thrombotic tendency in MPDs, by ETP parameters, did not emerge in this study except the increase of ETP in ET compared to the control group. Increased ETP values were characteristically observed in low PT/APTT group. The automated ETP test can play an important role in the evaluation of haemostatic function in patients in a hypercoagulable state. A potential clinical implication of these findings is that the laboratory investigation of the coagulation function, presently performed with the PT and APTT, is inadequate to assess the true risk of thrombosis. Perhaps the measurement of thrombin generation might be more suitable to evaluate the thrombotic risk. Although plausible, this hypothesis needs to be sustained clinically.

Table 1.

	CONTROL	AMI	MM	MPD	LOW PT/APTT
tlagsec	19.2	41.4	24	29	29
tmax sec	54	86.2	91	77	75
CmaxmA/min	123.5	124.2	94.1	95.2	115.1
ETPmA	395	352.6	370	358	434

1907

COMPARATIVE STUDY OF CORRELATION D-DIMER WITH THE TYPE OF NEOPLASM AND THE PRESENCE OR NOT METASTASIS IN PEOPLE WITH TUMOR AND IN THE GENERAL POPULATION

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Background and Aims. To study the values of d-dimer in neoplastic patients, both in relation to the type of tumor, and the presence or absence of metastases, and compare our results with those in the general population, because as we know, the d-dimer, resulting from the breakdown of fibrin, an indicator of activation of fibrinolysis and increased not only in thrombosis and pulmonary embolism, but also in cases of malignancy. **Materials and Methods.** We studied a total of 82 cancer patients (39 men and 43 women), average age 70. 4 years. Everyone was measured with the help of an automatic analyzer using the immunotholosimetrias levels of d-dimer (the lowest price was set at 250 ng /ml), while the results were compared with the group of 80 people (normal controls), roughly similar age (average: 65. 8 years). Followed by a statistical study of our data using the statistical package SPSS. **Results.** The average rate of d-dimer was: 1715 ng /ml in 11 cases of endometrial cancer (of which 6 with metastases), 1498 ng /ml in 19 women with breast cancer (11 with metastases), 1192 ng / ml in 12 patients with ovarian cancer (7 with metastases), 545 ng /ml for 22 patients with lung Ca (12 with metastases) and 512 ng /ml for those with Ca colon (18 patients of which 5 with metastases). Similarly, in the control group the average rate was 217 ng /ml. **Conclusions.** Therefore: 1)The average rate of d-dimer in all forms of cancer are greatly increased, compared with the general population. 2)The higher values observed in endometrial cancer and breast cancer, and 3)In all forms of cancers, no statistically significant correlation exists between the presence of metastases and increased d-dimers.

1908

BLOW-UP PLASMIN GENERATION: A THEORETICAL STUDY OF THE FIBRINOLYTIC REGULATORY SYSTEM

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Blood coagulation system (BCS) is responsible for thrombi formation and bleeding stoppage, while fibrinolytic system (FS) is responsible for the dissolution of thrombi and recovery of blood supply. Under normal physiological conditions, there is a dynamic balance between these two systems. A disruption of this balance may lead to thromboses and embolism or, on the contrary, to bleeding and blood loss. Therefore the investigation of the regulatory mechanisms governing these two systems certainly is a matter of great scientific and practical interest. According to modern views, the processes of thrombolysis under normal physiologic conditions develop relatively slowly in comparison with thrombi formation processes. However, some of the clinically observed phenomena (such as the disseminated intravascular blood coagulation (DIC)) show that besides the processes of gradual clots dissolution, a rapid thrombi lysis may also occur in the organism. Obviously, a possibility to control the activation of such rapid fibrinolysis is a matter of great clinical interest, both for the purposes of thrombosis treatment and blood loss prevention. The aim of the present work was to reveal the possible mechanisms that could lead to the switching of the regulatory system to rapid thrombolysis. For this purpose we analyzed the network of kinetic reactions involved in the regulation of fibrinolysis. Summarizing the available scientific data, we derived a detailed graph-scheme representing the interaction of key substances such as plasminogen, plasmin, prourokinase, urokinase, tissue plasminogen activator, fibrin, prekallikrein, kallikrein, XI and XII factors of BCS. Analysis of the positive feedback loops existing in this network of reactions enabled us to distinguish a block of reactions forming the central catalytic core, that is responsible for the eventual autocatalytic generation of plasmin and urokinase. After the subsequent reduction of the detailed graph-scheme we eventually derived a mathematical model describing the kinetics of FS regulatory processes in terms of differential equations. Using the obtained mathematical model we investigated the problem of existence and stability of stationary states of the fibrinolytic regulatory system. It was established that within a wide range of parameters the system has a ground state, characterized by relatively small concentrations of active enzymes, and stable with respect to subcritical perturbations of active enzymes concentra-

tions. On the other hand, supercritical perturbations exceeding the activation threshold value lead to abrupt (blow-up) generation of plasmin in the system. Thus the proposed model offers an explanation of rapid-evolving fibrinolytic processes as a result of threshold activation of blow-up plasmin generation. Parametric diagrams of stability of the ground state were built. It was shown that blow-up generation of plasmin may be caused not only by a supercritical perturbation of active enzyme concentrations, but also by the change of system parameters leading to a loss of stability of the ground state. We hope that the results obtained in this work will be of interest not only to researchers specializing in the investigation of FS regulation, but also to specialists in clinical hematology.

1909

DISTRIBUTION OF THE CEREBRAL VENOUS SINUS THROMBOSIS IN GRAN CANARIA UNIVERSITY HOSPITAL DR. NEGRIN

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Background. Cerebral venous sinus thrombosis (CVST) is a rare disease of the central nervous system that represents 0. 5% of all the strokes. This disease is associated with an overall mortality of 9%. It usually affects young adults with acquired or congenital thrombotic risk factors. Clinical manifestations are produced by two basic mechanisms: thrombosis of the brain veins and sinus causing intracranial hypertension, infarction and edema. The diagnosis is made using imaging techniques such as CT-SCAN and MRI, in young patients showing typical clinical manifestations. **Aims.** Revision of the patients diagnosed of CVST from the year 2000 to nowadays, evaluating their clinical and biological conditions. **Results.** 24 patients with ages from 20 to 77 were diagnosed of CVST using imaging techniques: 17 women (70 %) and 7 men (30 %). 60 % of the events were localized in the superior longitudinal sinus. In 30 % of cases, there was more than one affected sinus. 20 (84 %) patients had a screening thrombophilia test performed which included: resistance to activated C protein, molecular study of Factor V Leyden and G20210 mutation., study of the inhibitors (AT, PrC, PrS), fibrinolysis proteins (PLAT, PAI1f and plasminogen) lupic anticoagulant, anti cardiolipin antibodies, anti beta 2 glycoprotein and serum homocysteine levels; finding some kind of alteration in 10 (50%) of these patients. (Table 1). The risk factors that were found were: the intake of oral contraceptive medication, obesity, sedentarism, puerperium, smoking, collagenosis and neoplasia. Of the 7 patients who were taking oral contraceptive pills, 3 presented a FII G20210 mutation. One of these patients presented also high levels of homocysteine. Most of the patients begun therapy with heparin and anti vitamin K medication, without observing recurrence of the event. **Conclusions.** CVST is a disease etiologically related mainly with infectious processes, but nowadays it is considered secondary to a state of hypercoagulability in young adults, associated to a confirmed thrombophilic state. Its outcome, sometimes fatal, considerably improves with a prompt diagnosis followed by an early anticoagulant treatment. Our results correlate with the limited existing bibliography, showing a predominance of the feminine sex and the affected sinus. We have found biological trombophilic alterations in 10 patients out the 20 that were analyzed, and also vascular risk factors, being the most frequent one the intake of contraceptive oral medication. The duration of the anticoagulant treatment is controversial, coming from 6 months up to indefinite; depending on the observed procoagulant alterations, the severity of the clinical manifestations, recurrence and the persistence of the vascular risk factors. The relation between the evolution and early treatment, makes it necessary the clinical suspect in young women with associated risk factors and headache, who come to the emergency service.

Table 1.

Congenital alterations	N. of patients (%)	Acquired alterations	N. of patients (%)
Heterozygous state for FIIG20210*	3 (27%)	Hyperhomocysteinemia*	4 (36%)
Protein S deficit	1 (10%)	Lupic anticoagulant	3 (27%)

*1 patient showed both alterations

1910

THROMBOTIC MICROANGIOPATHY WITH SEVERE RENAL FAILURE SECONDARY TO BETA-INTERFERON TREATMENT FOR MULTIPLE SCLEROSIS

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Background. Thrombotic microangiopathies (TMA) are a heterogeneous group of diseases characterized by a mechanic hemolytic anemia, platelet consumption, and organ failure due to endothelial injury and development of microthrombi. Some TMA are due to a severe defect in ADAMTS 13, a plasma enzyme involved in the cleavage of von Willebrand factor (VWF) multimers into smaller and less adhesive VWF forms. ADAMTS 13 deficiency can either be hereditary or acquired due to autoantibodies that alter the protein function. Such secondary forms can be caused by drugs, pregnancy, bone marrow transplantation, HIV infection, systemic lupus erythematosus, or malignancy. In the literature, alpha-interferon is known to induce autoimmune manifestations including cases of alpha-interferon-induced TMA due to ADAMTS 13 autoantibodies. Although, beta-interferon can induce local reactions, no systemic autoimmune manifestations were reported. We report herein the first case of TMA due to ADAMTS 13 autoantibodies in a patient receiving beta-interferon treatment. Case report: A 52 years old male patient with multiple sclerosis treated since 2008 with beta-interferon was admitted to the emergency for dyspnea. The patient had no other medical conditions and received no other treatment. Renal function was normal a few months before admission. For two weeks preceding his admission, he was experiencing severe headaches due to severe arterial hypertension which was resistant to standard treatment. On admission, physical examination showed severe arterial hypertension (185/114 mmHg) associated with tachycardia (110/min) and signs of diffuse hyperhydration. Dipstick showed major proteinuria and microscopic hematuria. Biological investigations confirmed TMA with hemolytic anemia (hemoglobin at 5. 9 g/dL, LDH at 6N, low haptoglobin levels and elevated reticulocyte count) and thrombopenia at 75 G/L with severe anuric renal failure (creatininemia at 1131 µmol/L, uremia at 35. 5 mmol/L). Urinary analysis revealed proteinuria of glomerular profile (1. 60 g/day). Emergency hemodialysis was undertaken and plasma exchanges associated with plasma infusions were started promptly. Immunological investigations found an undetectable ADAMTS 13 activity due to a circulating anti-ADAMTS 13 antibody. There was no complement activation and antinuclear antibodies were present. Renal biopsy confirmed the diagnosis with severe arteriolar and glomerular TMA involving 50% of the glomerulus. After eight sessions of plasma exchanges associated with plasmatherapy, the hemolytic process could be stopped and plasmatherapy was discontinued. Meanwhile, steroids were introduced at the dose of 1 mg/kg/day and beta-interferon treatment was discontinued in the hypothesis of an autoimmune process secondary to beta-interferon treatment. There was no renal recovery and patient remained hemodialysis-dependent. During follow-up, ADAMTS 13 activity was still very low associated with recurrence of hemolysis and thrombopenia leading us to initiate rituximab treatment in addition to plasma exchanges associated with plasma infusions. **Conclusions.** This case demonstrates that beta-interferon treatment can be responsible for anti-ADAMTS 13 antibody-induced TMA and severe renal failure.

1911

PROTROMBOTIC COAGULATION ABNORMALITIES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA BEFORE AND AFTER START OF THE THERAPY

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Background. Patients with multiple myeloma (MM) have an increased risk of thromboembolic complications. The pathogenic mechanism of the increased activity of FVIII is unclear, but may be explained by neovascularization in the bone marrow stroma. It is known that the chemotherapy causes the activation of blood coagulation in myeloma patients. Extremely high level of FVIII may be a sign of hypercoagulation and risk factor of cardiovascular disease. **Aims.** To investigate activity of FVIII before and after start of the treatment in patients with newly diagnosed MM. **Methods.** We investigated platelet-poor plasma from 25 patients with newly diagnosed MM (age 51-78); 13(52%) persons had the primary thrombotic history and 12(48%) did not have thrombotic anamnesis. Control group comprised 82 ages-, sex-matched healthy individuals. The FVIII activity was measured before and after start of induction therapy. Differences were evaluated with non-parametric statistical methods (Wilcoxon test, Statistica 6. 0) and significance assumed for p< 0, 05. **Results.** FVIII activity in patients without thrombotic history did not differ from the control group before start of the therapy. However, it significantly increased during the induction treatment. FVIII activity in patients with primary thrombotic history was higher compared to the control group in both periods (Table 1). **Summary:** FVIII activity increased in patients with newly diagnosed multiple myeloma, especially in the group with primary thrombotic history. Activity of FVIII in patients without thrombotic history was significantly higher then the patients with it after start of the treatment.

Table 1.

Patients	Without thrombotic history (n=12)	With thrombotic history (n=13)
Laboratory parameters	FVIII:C (%) Me	FVIII:C (%) Me
Before therapy	166,0	240,5*
After start of therapy	264,0*,**	198,0*

*- p< 0,05 for difference with controls** - p< 0,05 for difference with values before therapy.

1912

SUCCESSFUL USE OF FONDAPARINUX IN A CHILD WITH HEPARIN-INDUCED THROMBOCYTOPENIA, RELATED TO ANTIPHOSPHOLIPID SYNDROME AND PROTHROMBIN G20210A MUTATION

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Background. Heparin induced thrombocytopenia (HIT) is an immune-mediated disorder characterized by the development of antibodies against the complex of platelet factor 4 (PF-4) and heparin. Documentation of antibodies against PF4-heparin complexes is necessary but not sufficient for diagnosis of HIT. When the diagnosis of HIT is confirmed or suspected, all forms of heparin should be discontinued and other non-heparin anticoagulants should be administered such as argatroban, bivaluridin, lepirudin. **Aims.** This case presentation aims that fondaparinux is an effective alternative treatment for children with HIT. We also emphasized some difficulties of pediatric thrombosis such as HIT and APS. **Case Report.** A 12 year-old-boy was developed deep vein thrombosis (DVT). Risk factors for initial thrombosis are antiphospholipid syndrome (APS) and heterozygous mutation for prothrombin G20210A mutation and methylenetetrahydrofolate reductase (MTHFR-C677T) gene. Anticoagulant therapy with warfarin for 12 months was effective, but discontinuation of warfarin after 12 months resulted in recurrence of DVT. Unfractioned heparin (UFH) was initiated during the acute period of DVT, but HIT was developed. Transition from unfractioned heparin to fondaparinux resulted in successful anticoagulation for a period of platelet recovery. **Conclusions.** This case highlights several difficulties in pediatric HIT cases. It is difficult to management of patients with HIT, especially in the presence of prothrombotic genetic risk factors and APS. The use of fondaparinux may be an alternative treatment modality for effective anticoagulation in the acute period of pediatric thrombosis in cases of HIT.

1913

EVALUATION OF THROMBOPHILIA IN WOMEN WITH RECURRENT PREGNANCY COMPLICATIONS

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Introduction. Inherited or acquired thrombophilia is associated with a high risk of pregnancy complications (preeclampsia, intrauterine fetal death, intrauterine growth restriction, pregnancy loss). Methylenetetrahydrofolate reductase (MTHFR) mutations, prothrombin G 20210A mutations, factor V Leiden G1691A and antiphospholipid syndrome cause thrombophilia. **Aims.** to evaluate the risk factor of thrombophilia in women with recurrent pregnancy complications. **Methods.** we studied 38 women with a history of recurrent pregnancy complications (median number of pregnancy complications: 3), age between 20 - 45 years, during a year (2011). Detection of thrombophilic mutations was made by Polymerase Chain Reaction and anticardiolipid antibodies by ELISA (IgG and IgM), with a confirmatory test of at least 12 weeks apart from the first measurement. **Results.** in 30 women (80%) diagnosis of thrombophilia was confirmed: twenty-one had MTHFR mutations (7 homozygous, 14 heterozygous), four had prothrombin G20210A mutation, in one case factor V Leiden was detected and in four cases anticardiolipid antibodies. In 40% of cases, other risk factors were associated (age > 40 years, smoking, obesity). **Conclusions.** Thrombophilia is associated with a high risk of pregnancy complications and a thrombophilic evaluation should be performed in women with recurrent pregnancy loss.

1914

A SURVEY OF THROMBOPHILIA RISK FACTORS AND MARKERS IN IRANIAN PATIENTS WITH DEEP VEIN THROMBOSIS

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Background. Thrombophilia is an abnormality of blood coagulation that can cause problems such as the deep-vein thrombosis (DVT). **Aims.** In this study we evaluated the effect of hereditary factors on thrombophilia. **Methods.** 95 DVT patients received heparin therapy followed by warfarin for six months. Then warfarin was stopped, and heparin therapy was continued for two more weeks. Thrombophilia factors and other markers were examined in these patients. **Results.** 37 patents (38. 9%) were male and 58 were (61. 1%) female. 18. 94% of patients had increased levels of homocysteine. 10. 52 % of patients had elevated levels of factor VIII. 21. 05% of patients had elevated levels of factor V-Leiden. **Concolusion**:DVT was most common in females. DVT was significantly associated with a history of thrombosis, OCP use and positive family history. Elevated levels of homocysteine and factor VIII, and S protein deficiency were common.

1915

USE OF FIBRINOGEN CONCENTRATE IN THE INFANT AND NEONATAL POPULATION

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Background. Acquired hypofibrinogenaemia can develop for a number of reasons including disseminated intravascular coagulation and massive transfusion and in the neonatal population, prematurity or congenital liver dysfunction are other possible causes. Fibrinogen concentrate (FC) was first studied in congenital hypofibrinogenaemia or dysfibrinogenaemia but in older children (6 years and up) and adults. There is little, if any, data available on administration in infants and neonates to guide dosage. In 2009, the Irish Blood Transfusion Service withdrew cryoprecipitate - made in Ireland- (except in some very well defined circumstances) and replaced it with fibrinogen concentrate for use in hypofibrinogenaemic states. This decision was made on the basis of a perceived lower risk of transmission of variant Creuzfeldt-Jaeger Disease with the latter product. Our institution is a tertiary referral paediatric centre which performs all neonatal cardiac surgery on the island of Ireland. **Aims.** To record the dose of FC given, clinical background/indication of patients receiving FC and increment of fibrinogen level found on laboratory testing. **Methods.** We identified the patients who received fibrinogen concentrate since its introduction through blood bank records. The notes were then procured and reviewed with respect to clinical background and exact dose of FC administered and weight of child. The dose per kilogram of body weight was calculated. Laboratory parameters prior to and following FC administration were recorded as were any other blood products administered. Not all patients had a repeat fibrinogen level done on the day they received FC so levels the following day were recorded as post dose levels. **Results.** We identified 49 patients under the age of 12 months who received fibrinogen concentrate amounting to 74 episodes as some had repeated infusions. 27/49 were neonates and they accounted for 40/74 transfusions. The recommended dose at our institution for fibrinogen concentrate is 70-100mg/kg. 9/74 episodes used doses lower than 70mg/kg and 6/74 doses higher than 100mg/kg. Data on weight was not obtained in 6/74. Fibrinogen levels prior to and following FC infusion where available were recorded. 1/74 did not have a baseline fibrinogen level. 4/74 did not have a fibrinogen level done within 24 hours of infusion. On 3/74 occasions fibrinogen level post infusion was lower than the baseline level despite receiving 70-100mg/kg FC. On one occasion, there was no change in level. All other patients' repeat fibrinogen levels were higher on repeat. The range of fibrinogen level increment varied from 0. 02 to 2. 71 (0. 13-1. 88 in neonatal group). Patients were grouped into cardiac, liver and other groups depending on the main issue necessitating the use of FC. 25/49 patients required cardiac surgery, 6/49 had liver disease and the remainder had varying pathologies. **Summary and Conclusions.** Whilst FC has been in use for many years, there is a remarkable paucity of data regarding its use in neonates and young children. Our findings suggest that whilst our recommended dose is effective for the

majority of patients, there are a substantial number who require higher or repeated doses and monitoring is required.

1916

PREDICTIVE SCORE FOR EFFECTIVE BLEEDING CONTROL USING RECOMBINANT ACTIVATED FACTOR VII IN PERIOPERATIVE NON-HEMOPHILIC PATIENTS

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Background. Recombinant activated factor VII (rFVIIa) has been increasingly used to control life-threatening bleeding following trauma or surgery. Although growing evidences from widespread rFVIIa use may define the efficacy and safety outcomes, there is currently no validated prediction score available to identify non-hemophilic patients undergoing surgery who should have benefits for the off-label use. **Aims.** The purpose of this study was to develop a predictive score based on preoperative parameters to predict effective bleeding control in non-hemophilic surgical patients received perioperative rFVIIa. **Methods.** Between 1 September 2004 and 31 December 2009, a retrospective study was conducted in Songklanagarind Hospital, an 853-bedded tertiary care hospital in southern Thailand. The primary outcome was efficacy of bleeding control defined as patients did not need surgical re-exploration for hemorrhage within 24 hours after rFVIIa administration while the definition of ineffective bleeding control was patients died or needed re-operation due to hemorrhage. The secondary outcomes were units of blood or blood component administered within 24 hours before and after the first dose of rFVIIa. Multivariate logistic regression analysis was performed to develop a new prediction score. The total score of each patient with effective bleeding control was included in a multiple logistic regression equation for conversion to the predictive index. The best cut-off was derived from the ROC curves. To facilitate the clinical use, the probability of effective bleeding control was calculated for each quartile predictive score. **Results.** A total of 320 bleeding episodes from 243 non-hemophilic patients underwent surgery were analyzed. Effective bleeding controls were demonstrated in 153 patients (63%). Mean age and preoperative routine laboratory results were similar in both effective and ineffective groups. The median rFVIIa doses were 87. 3 μg/kg (IQR; 65. 5, 109. 1) in the effective group and 93. 8 μg/kg (IQR; 73. 8, 118. 6) in the ineffective group (p = 0. 13). The overall in-hospital mortality rate was 40%: 41 patients (26. 8%) were in the effective group and 56 patients (62. 2%) were in the ineffective group (p < 0. 001). After rVIIa therapy, the units of blood and blood component transfusion was significantly lower in the effective group (data shown as median (IQR): packed red cell (3 (2,6) vs 6 (3,11), p<0. 001); fresh frozen plasma (4 (3,7) vs 7 (3,15), p<0. 001); platelet concentrate (4, (0,10) vs 10 (0,15), p<0. 001). Multivariate analysis identified four variables as independent predictors for effective bleeding control after rFVIIa administered: timing of rFVIIa administration, intraoperative blood loss, postoperative INR values, and postoperative total unit of platelets transfusion. Using the final regression model, the area under the ROC curve was 0. 844. The rFVIIa successful scores and the probability of effective bleeding control with rFVIIa were shown. (Table 1). **Summary.** The use of this new prediction score may support decision making to identify patients with high probability of effective bleeding control from rVIIa therapy.

Table 1. Prediction of effective bleeding control scores with rVIIa therapy.

Predictors	Categories	Scores
Timing of rFVIIa administered	Pre- or pre- and intra-operative Intra-operative Post-operative Intra- and post-operative	0 44 31 28
Intraoperative blood loss	Per 1,000 mL	-1
INR values after the first dose of rVIIa administered	Normal Prolonged No data	0 -14 -15
Total units of platelet transfusion after the first dose of rVIIa administered	Per unit	-0.6

Score ranges	Probability of effective bleeding control
Less than or equal to -10	10%
-10 to 6.0	50%
5.9 to 23	82%
More than 23	94%

1917

REJUVENATION TREATMENT OF STORED RED BLOOD CELLS PARTIALLY IMPROVES THEIR HEMODYNAMIC/MECHANICAL PROPERTIES

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Background. Routine cold-storage of blood units in the blood bank induces deterioration of red blood cell (RBC) flow-affecting properties (FP), specifically deformability, self-aggregability, and adherence to blood vessel wall endothelia cells (EC), as well as their mechanical and osmotic fragility. This phenomenon progresses with storage duration and becomes significant already at the second week of storage. Since stored RBC (St-RBC) with impaired FP induces circulatory disorders, the transfusion of RBC with impaired FP may introduce a risk to blood recipients. To reduce this risk, a post-storage treatment has been proposed, by incubation of stored (St-RBC) units with "rejuvenating"supplements (sodium pyruvate, inosine, adenine and sodium phosphates) prior to transfusion. Previous studies with *in vitro* rejuvenation treatment of St-RBC have shown improvement, to different degrees, in red cells ATP and 2,3-DPG levels, deformability, mechanical and osmotic fragility. However, these studies differed in the storage protocols, in particular in employing St-RBC units with or without pre-storage leukoreduction, while leukoreduction is practiced only in part of the blood banks, depending on the policy of specific country's health authority. **Aims.** The present study was undertaken to systematically examine the efficacy of rejuvenation in rectifying storage-induced damage to RBC stored without leukoreduction. **Methods.** Non-leukoreduced St-RBC were treated with rejuvenation solution ("Rejuvesol") and their adhesion to cultured human microvascular EC and deformability, as well as osmotic and mechanical fragility were determined using a cell flow-properties analyzer (CFA) which provides comprehensive characterization of RBC hemodynamic/mechanical properties in large RBC population as function of shear stress. Concomitantly, we determined the level of RBC surface phosphatidylserine (PS), a mediator of RBC/EC adhesion and RBC splenic clearance, as well as intracellular levels of free radicals and Ca^{++}. These measures were compared with those of the respective untreated St-RBC and of freshly-donated RBC (same unit, pair-test). **Results.** Cold storage induced strong elevation of St-RBC/EC adhesion, cell rigidity, osmotic and mechanic fragility, concomitantly with intracellular Ca^{++} and reactive oxygen species (ROS) level. Increasing RBC adhesion correlated with translocation of PS to RBC surface, a known mediator of RBC/EC adhesion. Post-storage rejuvenation of St-RBC partially reversed these measures, except for PS translocation (80% reversal) and the corresponding RBC/EC adhesion which was completely reversed to that of freshly-collected RBC. **Conclusions.** Previous studies have shown that the mechanical behavior of RBC is affected by a number of structural changes, among them surface area/volume ratio, membrane and skeleton condition (protein cross-linking), and surface charge. All these are irreversibly altered during RBC storage. In accordance with that the present study shows that deformability and fragility of not-leukoreduced St-RBC are only partially reversed by rejuvenation treatment. Since leukoreduction has been shown to decrease storage-induced damage, it is likely that rejuvenation of leukoreduced St-RBC would yield better results. On the other hand, this does not apply to RBC/EC adhesion, which is completely reversed by rejuvenation treatment even with not-leukoreduced St-RBC.

1918

EVALUATION OF IRON OVERLOAD AND CARDIAC FUNCTION BY T2* MAGNETIC RESONANCE IMAGING IN ACUTE LEUKAEMIA SURVIVORS

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Patients with acute leukaemia require supportive blood transfusions to facilitate potentially curative therapy. Consequently they are at risk of iron overload which can manifest as organ failure eg. heart, renal and liver. Additionally these patients are exposed to high dose anthracycline chemotherapy increasing the potential for late cardiac toxicity. Iron loading is most commonly assessed through serum ferritin levels. However it is established that serum ferritin correlates poorly with organ damage. There is increasing evidence that T2* magnetic resonance imaging (MRI) is able to accurately quantify myocardial and hepatic iron loading through non invasive means. It has additionally been proposed that T2* MRI can identify patients at risk of symptomatic heart failure, making it attractive method for assessing patients who have received anthracycline chemotherapy. A single centre retrospective audit was conducted in adults patients attending clinic between Jan 2010 and Dec 2010 who had

received intensive chemotherapy, with raised serum ferritin levels (>1000µg/l) to evaluate the use of T2* MRI scans. 16 patients (13 AML, 1 ALL and 2 AA) were available for evaluation. The age range at diagnosis was 18-70yrs (median 45yrs) and were between 2 and 10 years from treatment (median 6 yrs). T2* MRI studies were performed using a 1. 5T CMR (Avanto, Siemens). Myocardial T2*, hepatic T2* and LV function were measured. Myocardial iron loading (MIL) was catagorised according to the following results criteria; negative ≥ 20ms, mild 20-14 ms, moderate 14-10ms and severe <10ms. Liver iron loading (LIL) was catagorised as; negative ≤2mg/g) >6. 3ms, mild (2-5mg/g) 6. 3-2. 7ms, moderate (5-10mg/g) 2. 7-1. 4 and severe (>10mg/g) ≤1. 4ms. Patients' had received a median of 42 units of blood (range 11-81) and had a serum ferritin of between 1069µg/l and 6571µg/l. There was no correlation between the number of blood transfusions and serum ferritin level. T2* Cardiac MRI did not identify MIL in any patient (0/16). 10 patients (10/16 = 63%) had mild LIL, however no patients had moderate or severe LIL. There was no correlation between serum ferritin levels and the presence of LIL. 7/16 (44%) patients had an abnormal cardiac function. These were either grade 1 or 2 diastolic dysfunction (n=1 and 2 respectively) or impaired LV systolic function (n=4). 4 of these patients had known cardiac disease therefore identifying 3 patients, who had no other cardiac risk factors, with sub clinical cardiac dysfunction. Although there was no clear correlation between cumulative anthracycline dose and heart function, there was evidence of cardiac impairment in patients who had received even small amounts of anthracycline chemotherapy (range 95mg/m2 -362mg/m2). We have shown that patients who have successfully undergone intensive therapy for acute leukaemia are at risk of cardiac dysfunction as demonstrated by T2*, although we could find no evidence of iron loading as a contributing factor in this heavily transfused group of patients. Routine assessment with serum ferritin appears unnecessary. While hepatic iron loading does not appear to be a major problem in this population, T2* MRI is useful in assessing the severity of hepatic iron loading.

1919

STUDY OF RESIDUAL LYMPHOCYTES IMMUNOPHENOTYPE IN THE PHOTOCHEMICALY TREATED PLATELET CONCENTRATES TRANSFUSED TO ONCOHEMATOLOGICAL PARTIENTS

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Background. Contamination of platelet concentrates (PC) by viable donors lymphocytes can lead to transfusion-associated graft versus host disease. Photochemical treatment (PCT) of PC with amotosalen and long-wavelength ultraviolet A (UVA) has been developed to prevent transfusion complications, associated with pathogens and donors leukocytes. The mechanism of action of PCT is non-selective inactivation of pathogens DNA and RNA. **Aims.** The aim of this study was to evaluate the impact of PCT on residual lymphocytes immunophenotype. **Methods.** We used an experimental model of PC in the form of pooled random-donor PC, to perform investigation of residual lymphocytes subpopulations. 16 PC were obtained by the buffy coat method (five buffy coats were pooled) with centrifugation and were suspended in 65% platelet additive solution. PCT of PC were performed with 150µM amotosalen and 3,6 J/cm^2 UVA light. We studied four groups of samples obtained from each PC: 16 samples after PCT on Day 1 and 16 - on Day 5 of storage, 16 untreated samples on the same days. All samples were investigated by flow cytometry. Lymphocytes were identified by the binding of the PE Cy-7-conjugated anti-CD45 antibodies. Lymphocytes (CD45+ cells) were stained with a FITC-conjugated monoclonal antibodies against CD3 (T-lymphocytes), against CD19 (B-lymphocytes) and with PE-conjugated antibodies against CD16CD56 (NK-cells) for gating lymphocytes subpopulations. **Results.** The number of CD3+ cells in untreated samples on Day 1 was 59,06±8,75. PCT of PC was not lead to statistically significant changes (p=0,37) in the number of T-lymphocytes on Day 1. The same number of CD3+ cells (p=0,87) were detected in untreated samples on Day 5. There were not observed differences between PCTed samples on Day 5 and on Day 1 (p=0,68), and between PCTed samples on Day 5 compared with untreated samples on Day 1 (p=0,48). The percentage of B-lymphocytes and NK-cells in untreated samples on Day 1 were 20,3±7,79% and 12,86±4,39% respectively. The same number of CD19+ cells (p=0,17) and CD16+CD56+ (p=0,59) were detected in untreated samples on Day 5. The percentage of B-cells in PCTed PC remained unchanged (p=0,14) on Day 1, whereas the percentage of NK-cells tended to decrease (p=0,05). The results of comparative evaluation of PCTed samples stored for 1 and 5 days demonstrated no differences in the distribution of lymphocyte subpopulations (p=0,68 for B-cells and p=0,2 for NK-cells). The data of comparative analysis of lymphocyte subpopulations in PCTed samples on Day 5 and in untreated samples on Day 1 showed that percentage of CD16+CD56+ cells decreased (p=0,008), and the percentage of CD19+ cells increased (p=0,007) significantly. The results of the distribution of lymphocytes subpopulations presented in Table 1. **Summary and Conclusions.** Inactivation of residual lymphocytes genetic material with PCT of PC was not lead to lymphocytes death during 5 days of PC storage. PCT did not significantly alter the distribution of lymphocyte subpopulations although there appeared to be a modest selective depletion of CD16+CD56+ NK-cells. Thus, these results show that subpopulations of T- and B- lymphocytes are the most stable, while the NK-cells - the most sensitive to PCT.

Table 1. Lymphocytes subpopulations in platelet concentrates (*mean ± standard deviation).

Lymphocytes CD markers (M ± SD *, %)	Untreated PLT Day 1	Untreated PLT Day 5	PCTed PLT Day 1	PCTed PLT Day 5
T-lymphocytes (CD3+)	59,06±8,75	58,63±6,31	60,87±9,65	61,16±6,46
B-lymphocytes (CD19+)	20,3±7,79	21,77±7,33	22,83±5,52	23,94±6,74
NK-cells (CD16+CD56+)	12,86±4,39	13,47±6,46	10,07±3,8	9,36±3,65

1920

PLATELET GRANULE CONTENT RELEASE IN PLATELET CONCENTRATES DURING STORAGE

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Introduction. Platelet activation during storage of platelet concentrates (PC) is an important event and can affect the clinical effectiveness of platelet transfusions. Platelet activation can be estimated through the measurement of proteins specifically located in platelet granules. **Aims.** In this study we evaluate the effect of storage on the expression and release of hemostatic and angiogenic proteins in of PC obtained from buffy coats by standard procedures. **Methods.** 15 (n per blood group: 5 O, 6 A, 4 B) PC were prepared from pools of buffy coats at the Ospedali Riuniti of Bergamo and sampled the day of preparation (D0) and after 3 days of storage (D3). 11 PC (type O=5, type A=6) were prepared by the Fenwal system, and 4 PC (type B) by the Terumo TACSI system. Samples were centrifuged to separate platelets (PLT) from supernatants (S-CP), and both preparations were tested for antigenic levels of P-selectin, vWF (vWF:Ag), Thrombospondin (TSP-1) and VEGF by ELISA. vWF was also measured as activity (vWF:Act) by the collagen binding assay. **Results.** At D0, platelets of A group showed the highest levels of all the molecules analyzed, while B the lowest. The storage caused an overall reduction in the content of the molecules, which varied according to the type of molecule and blood group. Particularly, platelets of B group showed the greatest reductions over time for all the markers. The decrease in platelet granule content was associated to a parallel increase in the correspondent S-PC. P-selectin and VEGF levels showed the greatest increment (+195%), followed by TSP-1 (+86%) and vWF:Ag (+49%), while vWF:Act remained unchanged. In S-CP of B group we observed the greatest increments in TSP-1 (+113%), VEGF (+447%) and P-selectin (+238%) levels. **Conclusions.** At baseline, platelets from A group possess the highest levels of vWF, TSP-1 and VEGF. During storage, platelet degranulation caused an accumulation of these proteins in S- PC. PC of B group showed the highest increase of angiogenic molecules over time. The different preparation method of PC of group B might be responsible of the differences observed.

1921

PATIENT TIME BURDEN ASSOCIATED WITH RED BLOOD CELL TRANSFUSIONS IN CANCER PATIENTS RECEIVING CHEMOTHERAPY IN OUTPATIENT CENTERS IN FRANCE

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Background. Anaemia is a common complication in patients with cancer receiving chemotherapy. The European Cancer Anaemia Survey estimated that the incidence of anaemia among patients receiving chemotherapy ranges

from 62. 4% in gastrointestinal/colorectal cancer to 88. 3% in gynecological cancer. Anaemia in patients with cancer can be treated with red blood cell (RBC) transfusions. Patient time burden associated with a treatment is an important factor to consider when measuring the benefits and challenges of a therapy. To date, no research studies aimed at estimating the patient time burden of RBC transfusion to treat anaemia have been identified. **Aims.** The aim of this study is to estimate the patient time burden associated with outpatient RBC transfusion treatment indicated for chemotherapy induced-anaemia (CIA) in patients in France with non-myeloid malignancies. **Methods.** A retrospective chart review of patients with cancer receiving an RBC transfusion was conducted at 8 outpatient centers selected to represent standard of care in France. Patients with a diagnosis of non-myeloid malignancy who received an outpatient RBC transfusion indicated for CIA within a 3 month eligibility period from August 01, 2010 to October 31, 2010 were identified at each site. Patients were randomly selected and screened for study eligibility. Medical records that documented patient and treatment characteristics and time spent in the clinic during a patient's transfusion visit were used to quantify the outpatient RBC transfusion time. Total RBC transfusion time for one unique transfusion visit per patient was measured as: time elapsed from pre- to post-transfusion vital sign assessment and from transfusion start to stop time. Additional data were also collected, including: elapsed time from haemoglobin (Hb) level testing to transfusion, elapsed time from blood draw for compatibility testing to transfusion; estimated transfusion day travel time and distance calculated with mapping software on the basis of patient address information; and clinical and demographic information. **Results.** A total of 128 patients (60. 9% male, mean age 64. 0 years [standard deviation: 12. 8]) were enrolled in the study. The four most frequent diagnoses were: lung cancer (25. 8%); gynecological cancer, (16. 4%); urological cancer (14. 8%); and gastrointestinal/colorectal cancer (14. 8%). The mean elapsed time between pre- and post-vital sign assessment was 3. 8 hours (95% CI: 2. 0-5. 6), including a mean of 3. 2 hours (95% CI: 2. 4-4. 0) spent on the transfusion itself. Hb level testing (mean pre-transfusion Hb level: 7. 9 g/dL [standard deviation: 0. 8]) and blood draw for compatibility testing were completed in a mean of 26. 1 hours (95% CI: 5. 1-47. 1) and 9. 9 hours (95% CI: 1. 0-18. 9) prior to transfusion, respectively. Patient one-way mean travel time to the transfusion centre was 37. 7 minutes (95% CI: 32. 6-42. 9) and mean distance travelled was 28. 0 km (95% CI: 15. 3-40. 8). **Conclusions.** In France, the management of CIA is a time consuming activity for patients with cancer as they are required to travel to a specific clinic, undergo blood testing (including Hb and compatibility testing) and endure the transfusion procedure itself. This burden should be considered when deciding on an anaemia treatment preventing the need for transfusions. More time efficient treatment alternatives are clearly warranted.

1922

A RETROSPECTIVE ANALYSIS OF USE OF PROTHROMBIN COMPLEX CONCENTRATE FOR REVERSAL OF ANTICOAGULATION WITH WARFARIN IN A LARGE CENTRE

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Introduction. Prothrombin complex concentrate (PCC) is indicated for the emergency reversal of warfarin therapy in patients with major bleeding manifestations or requiring urgent surgical procedures and for coagulation defects in liver disease. PCC nominally contains blood coagulation factors II, VII, IX, X, protein C and protein S as active ingredients. It also contains sodium and heparin (100-250 IU per vial) as excipients. **Objective.** We performed a retrospective analysis of use of PCC in a tertiary hospital in order to ascertain its dose, indications for use, efficacy (INR before ad after PCC administration), use of other blood products and its safety. **Methods.** Forty-four patients who had received PCC over a 12 months period between December 2008 and January 2010 were identified from blood transfusion records. PCC was administered in accordance with hospital protocols. The median dose of PCC prescribed was 2000 IU (range 1000 - 3000 IU). The most common reasons for anticoagulation were identified to be atrial fibrillation (n=18) and venous thrombosis (n=13). PCC use was approved by haematology team in 7 cases. All patients were anticoagulated with warfarin except one. Most decisions to treat with PCC were from nephrology for renal transplant patients (27%) followed by medical (23%), surgical (23%) and emergency department (11%). International normalised ratio (INR) was measured before and after the administration of PCC. **Results.** 23 patients with bleeding (52%) and 21 non-bleeding patients (48%) received PCC. INR was measured and documented within one hour of PCC administration in 28 patients (61%) and within 24 hours in 41 patients (91%). Time to infusion from prescription was unknown in a significant number of patients (n=29) due to lack of documentation. Only 19 patients (43%) received vitamin K (15 of 23 bleeding patients and 4 of 21 non-bleeding patients) prior to PCC administration. INR was recorded to be >1. 5 in 43 patients (98%) before the administration of PCC. After PCC administration INR was <1. 5 in 34 patients (83%) within next 24 hours. Blood products were used sparingly (red cells to 16, plasma to 7, fibrinogen concentrate to 1 and platelets to 2 patients) within 48 hours of PCC administration. No serious adverse events were recorded. **Conclusions.** These results clearly demonstrated the efficacy and safety of PCC in patients requiring rapid warfarin reversal. There was considerable reduction in the use of other blood products during the 24 hour period after PCC administration. Partial or complete response was seen in 37 patients (84%). The data also identified a significant number of patients who did not receive concurrent vitamin K (57%). In a significant number of patients (n=16) INR was not measured within one hour of PCC administration. The monitoring of INR within one hour and 24 hours of PCC administration is recommended to ensure sustained and effective reversal of warfarin. This may highlight an ongoing educational need.

1923

PREGNANCY IN SICKLE CELL DISEASE: MATERNAL AND FETAL OUTCOMES IN OUR PATIENTS RECEIVING PROPHYLACTIC BLOOD EXCHANGE TRANSFUSIONS

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Background. Sickle cell disease (SCD) encompasses inherited anemias due to beta globin mutations that result in the formation of sickled red cells and increased red cell turnover. The complications of SCD are both acute and chronic, and this combination results in significant morbidity, high healthcare utilization over the lifespan, and increased premature death. Whereas many avenues have been explored to manage complications of SCD as they arise, renewed attention has been focused on pregnancy management because during pregnancy the risk for preeclampsia and deep venous thrombosis is increased and occlusion of placenta blood vessels with rigid deformed erythrocytes can cause repeated miscarriages and intra-uterine fetal death. Blood exchange transfusion can prevent these complications by reducing the concentration of abnormal hemoglobin S. **Aims.** To describe perinatal and maternal outcomes for pregnant women with sickle cell disease (SCD) receiving prophylactic blood exchange transfusions. **Methods.** This was a retrospective cohort study, covering the period from January 2001 to December 2011, which included all pregnant women with SCD followed up at our institution inSicily. We managed 12 pregnancies in 10 patients (7 SS, 3 S/b-thalassemia) that were in care because of a history of severe sickling complications. 3 Patients had a history of one or more pregnancies with severe maternofetal complications when treated in other institutions without receiving prophylactic blood exchange transfusion. In our institution all the patients received manually or automated Red cell exchange using a Spectra-Cobe blood cell separator when the haemoglobin S (HbS) was more than >50% and the aim was to achieve a proportion of HbS below 30% and a hemoglobin level between 9 and 11 g/dL. The maternal and perinatal outcomes were compared to those of the same pregnant women when treated in other institutions not receiving prophylactic blood exchange transfusion. **Results.** We performed 40 automated and 24 manually red cell exchange. No serious maternal complication was observed, no fetal or perinatal death occurred and no low birth weight. **Summary and Conclusions.** Our study suggests that women with severe sickle cell disease, even if they have a serious obstetrical history, can carry their pregnancy to term, without major obstetric complications, through a combination of early management (first trimester) by a multidisciplinary team and a suitable policy of prophylactic manually or automated red cell exchange transfusion

1924

CLINICAL OUTCOMES OF ABO INCOMPATIBLE RBC TRANSFUSIONS

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Background. ABO mismatch blood transfusion is one of seriousness events related to blood components administration. **Aims.** We performed a retrospective review of ABO-incompatible RBC transfusions from our institution during four years (2007-2011). **Results.** Within the period of observation, 20 ABO mismatches were detected (effective transfusions and near miss events). 12 ABO mismatches were detected when blood samples are studied for pre-transfusions tests (mistake in sample collection or identification and one case of usurpation of identity). Of 8 patients who received more than 50 ml of incompatible blood, 4 (50%) manifested signs or symptoms related to the incompatible transfusion but none died. Hypotension, hemoglobinuria, postoperative bleeding were detected in survivors. In the half of our cases (4 patients), there were no associated signs or symptoms due to the incompatible transfusions.

Conclusions. ABO incompatible RBC transfusion does not inevitably mean death or even occurrence of symptoms. Awareness of staff at the bedside and laboratory of the potential for errors is one of the most effective tools for detecting and preventing transfusion errors.

1925

TRANSFUSION RELATED PULMONARY COMPLICATIONS: A SINGLE CENTRE EXPERIENCE

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Background. Transfused related acute lung injury (TRALI) is a major cause of transfusion related morbidity and mortality. Its real incidence is unknown because is an underdiagnosed entity and remains a challenge to the practicing clinician. An immune mechanism is recognized in most cases of TRALI, related with anti-HLA class I and class II or anti-HNA antibodies in donor´s plasma. Non-immune mechanisms include the age of stored blood, neutrophil-priming agents released in stored cellular blood components and bioactive lipids. **Aims.** To review the experience on Transfusion related pulmonary complications in the last 12 years in a tertiary-care teaching hospital. MATERIALS AND **Methods.** Transfusion reactions notified between January 2000 and December 2011 were reviewed. Cases of transfusion related respiratory symptoms, including dyspnoea and desaturation, were selected. Age, sex, diagnosis, clinical course and outcome, type of blood component and age of product at time of transfusion were reviewed. Clinical charts were reviewed by three independent observers and a consensus diagnosis and degree of imputability was assigned. **Results.** 64. 559 patients were transfused in the last 12 years in the Hospital Universitario Central de Asturias. 288. 599 blood components were administered. 554 transfusion reactions were notified. In 40 patients (7. 2%) respiratory symptoms were present. A chest-X ray was performed in only 10 patients. No serologic studies were performed. The median age was 62 years (range 21-73), 38 patients (95%) were older than 50 years; 22 were female (55%). Packed red blood cells (all of them leucorreduced) were the most commonly blood component associated with transfusion related respiratory symptoms in our Centre, 25 cases (62. 5%9, followed by platelets, 11 cases (27. 5%) and fresh frozen plasma, 4 cases (10%). Packed red blood cells involved in transfusion reactions had more than 14 days of storage in 30 cases (75%). The most frequent diagnosis was hematologic malignancy, during the induction phase of chemotherapy, 26 patients (65%), followed by cardiac surgery, 8 patients (20%). Only 3 patients (7. 5%) were defined as possible TRALI in according to degree of imputability. No deaths were attributed to TRALI. **Conclusions.** Real incidence of transfusion related acute lung injury, which frequently is not recognized or misdiagnosed, has not been stablished. It´s important an adequate clinical diagnosis, including x-Ray and serologic studies, in order to stablish appropriate therapeutic measures and effective preventive measures. Hematologic malignancies, cardiac surgery and prolonged storage time of packed red blood cells were associated with transfused related respiratory symptoms, as previously reported in the literature.

1926

PRBC MEMBRAINE MECHANICAL FRAGILITY - SOURCES OF VARIABILITY IN A BLOOD BANK INVENTIORY

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Background. Red Blood Cell (RBC) membrane Mechanical Fragility (MF) had been proposed as a candidate metric of storage lesion with potential relevance to cells' performance post-transfusion *in-vivo*. While a number of studies demonstrated loss of RBC deformability/increase of fragility during cells' storage, the clinical relevance of this effect remains to be proven. Focus on storage time, however, typically ignores variability in pRBC due to donor-to-donor differences, as well as due to differences in manufacturing processes and storage practices. These, largely storage time-independent confounding factors, can significantly impact the properties of pRBC membrane in blood bank inventories which can potentially result in a variable in-vivo performance of cells even of the same storage age. **Aims.** To determine the impact, if any, of donor-related and manufacturing-related variables on pRBC properties in blood bank inventory. **Methods.** 102 pRBC samples, all irradiated, prepared by either Whole Blood donation (WBD) or through apheresis collection (AC) and leukoreduced either at 4-6 ºC or at room temperature (Warm Leukoreduction, WL), were collected from 4 test segments, combined, and characterized by storage time (AGE), total hemoglobin (HBT), auto hemolysis (AH), blood type, and donor demographics. To obtain variable-parameter fragility profiles, mechanical stress was applied using a bead mill with the oscillation frequency held at 50Hz while durations were varied between 0. 5 and 45 minutes. Fragility profiles were described in terms of hemolysis levels at particular durations (H), the inverse thereof (S), and the slope of the fragility profile curve (K). **Results.** MF parameters of pRBC varied significantly between the units with differences reaching 100-fold for selected parameters. Manufacturing variables were found to be the most significant predictor of MF accounting for 10 to 40 percent of the observed variability. Manufacturing-related aspects including the type of blood collection and leukofiltration, blood bag types, storage solutions and in-bag hemoglobin concentration were tightly cross correlated resulting in uncertainty in the determination of the underlying cause of variability. Donor demographic, including donor age, hemoglobin at donation, gender and race were poorly correlated with MF. AGE and time since irradiation were similarly poorly correlated with MF likely due to their contribution being substantially smaller than variability due to manufacturing and donor-specific differences. Unaccounted for variability in MF parameters is likely due to donor-to-donor variability due to varying cells' biological age at donation and potential individual donor differences in membrane composition. **Conclusions.** Donor-to-donor differences are indicated as a single most significant factor responcible for MF variability-Cold leukoreduction and/or apheresis collection may be preferable manufacturing approaches for preserving pRBC plasticity, although further work will be needed to account for confounding effects. Optimization of manufacturing practices has the potential to significantly impact in-vitro MF of pRBC in storage. **Disclosures.** This work was supported in part by Blaze Medical Devices of Ann Arbor, MI.

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